UC Santa Barbara
UC Santa Barbara Previously Published Works

Title

Strain differences in maternal neuroendocrine and behavioral responses to stress and the relation to offspring cocaine responsiveness

Permalink

https://escholarship.org/uc/item/2n1765tk

Journal

International Journal of Developmental Neuroscience, 78(1)

ISSN

0736-5748

Authors

Bagley, Jared R
Adams, Julia
Bozadjian, Rachel V
et al.

Publication Date

2019-11-01

DOI

10.1016/j.ijdevneu.2019.06.009

Peer reviewed

Strain differences in maternal neuroendocrine and behavioral responses to stress and the relation to offspring cocaine responsiveness.

Jared R. Bagley[1], **Julia Adams**[1], **Rachel V. Bozadjian**[1], **Lana Bubalo**[1], **Tod E. Kippin**[1,2,3,4]

[1]Department of Psychological & Brain Sciences, University of California Santa Barbara, Santa Barbara, CA, 93106

[2]Neuroscience Research Institute, University of California Santa Barbara, Santa Barbara, CA, 93106

[3]Department of Molecular Cellular and Developmental Biology, University of California Santa Barbara, Santa Barbara, CA, 93106

[4]Institute for Collaborative Biotechnologies. University of California Santa Barbara, Santa Barbara, CA, 93106

Abstract

Early life stress exposure, including prenatal stress (PNS), influences subsequent risk for many disorders, including substance abuse, and these effects interact with genetic factors to determine risk for disease. We previously demonstrated gene X environmental interactions across the BXD recombinant inbred mouse strain panel and their progenitor strains in PNS modulation of cocaine-induced reward and locomotion. Critical to dissecting genetic interactions with PNS is consideration of the modes of stress transmission to the offspring. Both maternal neuroendocrine responses during stress and subsequent maternal-offspring interactions following stress may serve as transmission modes for PNS-induced changes in cocaine responsiveness. Therefore, we characterized the maternal stress response by measuring restraint stress-induced plasma corticosterone (CORT) during gestation as well as effects of restraint stress on dam-pup contact in the first 10 postnatal days in BXD and progenitor mouse strains. Restraint stress interacted with strain to affect plasma CORT levels and dam-pup contact, indicating heritable variation of the maternal stress response. Furthermore, strain-level variance in maternal stress response correlated to the impact on cocaine response exhibited by adult offspring. These findings implicate multiple modes of maternal stress response in alterations of offspring drug responsiveness and indicate that assessment of maternal endocrine and behavioral responses during early life can be utilized to dissect the complex intersection of maternal factors, the response of the offspring and genetics.

Corresponding Author: Jared Bagley, Department of Psychology, State University of New York at Binghamton, Binghamton NY, 13902, jbagley@binghamton.edu, Phone: (805) 448-1399.

Keywords

prenatal stress; cocaine; addiction; maternal behavior; glucocorticoid; genetics

---

Cocaine use disorder, and drug use disorders generally, have a profound impact on afflicted individuals which results in a substantial cost to society [1]. Cocaine use disorder has a complex etiology, with genetic and environmental factors contributing both independently and through interactions that render some vulnerable to develop dysregulated use of cocaine. Early life factors may be particularly impactful due to the vulnerability of developing tissue to insults that cause long-lasting alterations in development. Identifying these factors, their interactions, and subsequent neurobiological mediators will facilitate discovery of improved treatment and preventative methods.

Early life stress, during both prenatal and early postnatal periods, is implicated in subsequent risk for drug use disorders [2]. Early life stress exposure can have developmental programming effects that increase intake and preference for cocaine later in life [3–6]. Furthermore, the effects of early life stress exposure are modulated by genetic background, suggesting that stress exposure interacts with genetic variants. Ultimately, cocaine abuse risk may only be fully accounted for by understanding these interactions. We have investigated the impact of genetic background on prenatal stress (PNS) by comparing the effects of PNS on cocaine-induced locomotor behavior and cocaine reward in the C57BL/6J (B6) and DBA/2J (D2) inbred mouse strains. Differential effects of PNS across these mouse strains indicate that genetic variants between the strains are modulating the effects of PNS [7]. In order to dissect these genetic by early environment interactions, we utilized the BXD recombinant inbred panel, derived from the B6 and D2 strains, to perform quantitative trait locus (QTL) mapping and genetic correlation analysis with other phenotypes, including gene expression, to nominate candidate genes that may interact with PNS to affect cocaine response [8]. Critical to interpreting the effects of these genes and pursuing further investigation is consideration of the modes of PNS transmission to the offspring.

Glucocorticoids are a likely mechanism of stress transmission from mother to fetus. Environmental stressors cause a response of the HPA axis that ultimately results in an increase of plasma glucocorticoid levels [9]. PNS procedures are repeatedly shown to cause increases in maternal plasma corticosterone (CORT) levels which is the primary glucocorticoid in rodents [10–14]. Although the placenta may act as a metabolic barrier to attenuate glucocorticoid transfer, it is not an absolute endocrine barrier, as fetal CORT levels rise after maternal stress exposure [11,13]. It is this in-utero exposure to elevated glucocorticoids that may explain the PNS phenotype in adult offspring. Inhibiting the dam CORT response by adrenalectomy attenuates or eliminates many of the effects of PNS on the offspring [14–16]. And exogenous administration of glucocorticoids during pregnancy produces similar effects to PNS, including altered responses to psychostimulants, morphine and ethanol [15–17]. There are reported differences in the CORT stress response in un-pregnant mice of the B6, D2 and BXD strains [18–20]. Given this evidence, the maternal CORT response to stress may vary across the BXD strains and may partially or fully account for the strain differences in the effect of PNS on cocaine responsiveness.

In addition to effects on plasma CORT levels, PNS procedures are also known to alter the maternal behavior of the stressed dam, thus altering post-natal environment of the offspring and potentially contributing to PNS-induced changes in cocaine responsiveness. The impact of PNS on maternal behavior has received limited study in mice with mixed result. A single traumatic experience on G12 reduced licking and grooming by natural mothers [21]. Other studies report no changes in nurturing behaviors but increases in aggression of stressed dams towards intruders [22–24] However, in rats, PNS has been widely reported to reduce licking and grooming of the pups [25–29], reduce time nursing [29] and to increase latency to retrieve, with lower retrieval rates of pups, when they are separated from the dams [27].

Both mouse and rat pups are dependent on maternal care, and the development of pups can be altered by variation in maternal behavior. Licking and grooming by rat dams negatively correlates with HPA axis reactivity in adult offspring [30,31]. Maternal behavior may also affect cocaine responsiveness. Manipulations that increase licking/grooming frequency, including temporary maternal separation and litter size reduction, cause decreased locomotor sensitization and CPP in male but not female rats and decreased cocaine self-administration (only males tested) [32–34]. Thus, there is compelling evidence from rodent studies indicating that PNS may alter maternal behavior, and variation in maternal behavior can have long-standing consequences for the offspring, including addiction-related responses. However, it is unclear if genetics modulate PNS-induced alternations in maternal response, and given evidence for altered stress response between inbred mouse strains [35–38], the BXD panel provides an ideal avenue to investigate both genetic contributions to PNS-induced altered maternal-pup interactions as well as the relation of these alterations to subsequent drug responsiveness.

In order to dissect genetic contributions to putative early life modes of PNS-induced perturbations, we employed the BXD recombinant inbred panel of mice to examine maternal endocrine response during pregnancy and postnatal maternal behavior. The maternal endocrine response was assessed by measuring plasma CORT levels measured pre- and post-stress during pregnancy. Maternal behavior was indexed in control and PNS dams by measuring the frequency of dam-pup contact during the first 10 postnatal days. Strain differences in the CORT response to stress and strain differences in the effects of stress on dam-pup contact were tested for association with PNS effects on cocaine responsiveness exhibited by the offspring in adulthood (Bagley, Szumlinksi, Kippin, 2019).

Methods

Subjects

C57BL/6J, DBA/2J, BXD strains (n=21) (The Jackson Laboratory, Bar Harbor, MI) were housed in a temperature- and humidity-controlled vivarium on a 12-h light–dark cycle. All mice were maintained on *ad libitum* mouse chow and water access. All procedures were approved by the University of California at Santa Barbara (UCSB) Institutional Animal Care and Use Committee and conducted in accordance with the National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals [39].

Breeding

All mouse strains were purchased from the Jackson Laboratory to establish a breeding colony in UCSB facilities (see the x-axes of Figure 2 for strains used in the experiments). The offspring of this colony were used for timed breeding of females which were subsequently exposed to repeated restraint (see below) or not and that generated the offspring subjects for behavioral analyses. Adult males and females, at 8 to 24 weeks of age, were paired for four days. Pregnancy was confirmed by weight gain and the dams were assigned to PNS or control conditions. Females that failed to conceive were re-subjected to the breeding procedures in future cohorts. Impregnated females were only used to generate a single litter of offspring, which were used in behavioral experiments. Male breeders were used for multiple cohorts.

Maternal Restraint Stress

PNS was induced by subjecting pregnant mice to repeated restraint stress, two weeks following the initial breeding setup. This corresponded to embryonic day 11 through 14. The PNS dams were taken from the vivarium into the laboratory and were restrained in 50 mL conical tubes for 1 hour periods, three times a day (during the light phase). Each 1 hour stress session was separated by 1 hour of home cage access. Restraint continued daily until parturition. The control dams were left undisturbed in their home-cage and were not removed from the vivarium during pregnancy.

After parturition, PNS and control litters were left undisturbed with the dams. Litters were weaned at approximately 3 weeks of age and the sexes were housed separately. The weanlings were left undisturbed until behavioral testing.

Maternal Corticosterone Response

On stress day 1 and 5, blood was collected by puncturing the submandibular vein with a lancet, once immediately before the first restraint session and once immediately after the first session. Approximately 50 microliters was collected into lithium heparin-coated tubes and then centrifuged for collection of plasma. Samples were stored at −80 degrees until ready for processing.

Plasma corticosterone concentration was measured with the use of the DetectX® CORT Enzyme Immunoassay kit (ArborAssays K014-H5, Ann Arbor, MI, USA). Room temperature plasma samples were diluted 1:450 in assay buffer and processed according to the provided protocol. A microplate reader (Elx800, BioTEK, Highland Park, VT, USA) was used to measure optical density at 450 nm and plasma concentration was interpolated from a standard curve. Stress day 1 and 5 samples were assessed for the progenitor strains, and stress day 1 was assessed for the BXD strains. All samples were run in duplicate within a plate and the intra-assay reliably was assessed by the coefficient of variation for the duplicates. Ten samples were assessed on two independent plates to determine the inter-assay coefficient of variation. Pre and post-stress samples for a subject were always run on the same plate and strains were balanced between plates. Sample sizes: B6=12, D2=9. For BXD strains, sample size range/mean/mode was 4–7/6/6.

Maternal-Pup Contact

After parturition, dams and their litters were observed from postnatal day (PND) 1 through 10. Observations consisted of 4 sessions daily, at times 0930, 1300, 1700 and 2000 (lights on at 0700, lights off at 1900). Night vision goggles were used during the 2000 session so that the dark phase was not disrupted. Each session consisted of one observation every 3 minutes for a total of 5 observations. At each observation, dams were noted to be in contact or not in contact with the pups. The mice were observed in their home cage and in their home rack position. Care was taken not to move the cage and to reduce any noise made by the observer. In total, dams received 200 observations. The number of in-contact observations was divided by total observations received to quantify contact. Sample sizes: B6 PNS n=9 control=8, D2 PNS n=5 control n=6. For BXD strains, samples size range/mean/mode: PNS=2–13/7/7, control=2–12/7/7.

Inter-observer reliability was assessed by performing simultaneous observations with pairs of raters blinded to the other observer's observation.

Offspring Cocaine-Psychostimulant and Reward Response—The BXD offspring were assessed for cocaine place preference, as well as acute locomotor response to cocaine and locomotor sensitization. Animals (9 weeks of age, both sexes) were placed in a 2-chamber CPP apparatus (tracking by ANY-maze software, Stoelting) for a 15 minute pre-test, followed by daily alternating conditioning sessions. Conditioning sessions consisted of a saline or cocaine (10mg/kg) intraperitoneal injections immediately prior to placement in one of the two chambers without access to the other chamber. Conditioning started with saline on day one, and alternated saline/cocaine daily for a total of 4 saline and 4 cocaine sessions. On the day following the last conditioning session, mice were placed for a 15 minute post-test (access to both chambers, no injection). CPP was measured as a change in time in cocaine-paired side from pre-test to post-test. Acute locomotor response to cocaine was measured as distance traveled during the first cocaine conditioning session minus distance traveled in the first saline session and locomotor sensitization was measured as change in distance traveled between the fourth and first cocaine conditioning session. See Bagley et. al. 2019 for further details.

Statistics

Data are presented as mean ±SEM. Analysis of variance (ANOVA) was used for analysis of stress and strain effects. Heritability was assessed by partial eta squared. Strain level correlations were assessed between PNS effect on maternal CORT and dam-pup interaction and the PNS effects on offspring cocaine-related phenotypes. Where correlations were found, PNS effects on maternal CORT and dam-pup contact were utilized to adjust offspring strain means in order to re-evaluate QTL mapping results previously published by Bagley et. al. 2019. Two standard deviations from the mean outliers within strain were excluded, except for heritability calculations. The level of significance was set at $p < 0.05$.

Results

Corticosterone

In order to assess the effects of stress (change from baseline to post-restraint), stress-day and strain on plasma CORT, the progenitor strain data was subjected to a mixed-factorial ANOVA. A stress by strain interaction [$F(1,17)=12.6$, $p=0.002$], a day by strain interaction [$F(1,17)=6.4$, $p<0.001$], a main effect of day [$F(1,17)=37.6$, $p<0.001$] and a main effect stress [$F(1,17)=122.5$, $p<0.001$] were found.

A simple main effects tests for stress revealed an effect of stress in both strains ($p<0.05$), with both strains demonstrating an increase in CORT as a result of stress. However, B6 mice had greater CORT levels at baseline ($p<0.05$) and D2 mice had greater CORT levels post-stress ($p<0.05$), indicating the magnitude of the CORT stress response was greater in D2 mice. The magnitude of the CORT response was calculated as the difference from baseline to post-stress (within individuals) and assessed for strain and day effects. A main effect of strain [[$F(1, 17)=12.6$, $p=0.002$] was found (D2 greater), but no effect of day or strain by day interaction were found, indicating no habituation or sensitization to restraint stress from day 1 to 5.

Simple main effects of day revealed an effect of day in both strains ($p<0.001$), with both strains demonstrating on increase in CORT levels from day 1 to day 5. Given there was no evidence of a change in stress response across days in either strain, the stability of the baseline CORT levels in B6 and D2 mice were assessed by mixed 2-way ANOVA, with baseline on day 1 and 5 as within factor and strain as a between factor. A stress day by strain interaction [[$F(1, 19)=12.5$, $p=0.002$], a main effect of stress day [[$F(1, 18)=92.7$, $p<0.001$] and a main effect of strain [[$F(1, 18)=18.1$, $p<0.001$] were found. Simple main effects for stress day, within strain, revealed that both B6 and D2 strains increase baseline CORT levels from day 1 to 5 ($p<0.001$ for B6, $p=0.001$ for D2). The magnitude of this shift was greater in B6 mice [[$F(1, 18)=12.5$, $p=0.002$] (see figure 1).

The BXD strains were assessed by mixed 2-way ANOVA, with the baseline and post-stress CORT levels as a within factor and strain as a between factor. A stress by strain interaction [[$F(20, 109)=3.9$, $p<0.001$], a main effect of stress [[$F(1, 109)=254.6$, $p<0.001$] and a main effect of strain [[$F(1, 109)=3.0$, $p<0.001$] were found. Tests within baseline and post-stress revealed an effect of strain in both, [[$F(20,107)=1.9$, $p=0.021$] and [[$F(20,107)=3.8$, $p<0.001$] respectively (see figure 2a.). No correlation was detected for strains means at baseline to strain means post-stress ($r=0.31$, $p=0.156$), or strain means at baseline to the magnitude of CORT change due to stress ($r=0.14$, $p=0.53$), indicating basal strain CORT levels may have limited or no relationship to the CORT stress responses.

A 1-way ANOVA for the magnitude of the CORT change from baseline to post-stress revealed a strain effect [$F(20, 106)=3.3$, $p<0.001$], indicating heritability of the CORT stress response. The heritability estimate is 0.42.

The average intra-assay coefficient of variation for the CORT assay was 6.1% and the average inter-assay coefficient of variation was 15.1%.

Maternal Behavior

A 2-way ANOVA for maternal-pup contact in the progenitor strains revealed a main effect of strain [[$F(1, 24) = 4.5$, $p<0.044$], with D2 mice observed to have more contact with pups (see figure 2b). No significant main effect of PNS or significant interactions were found.

A 2–way ANOVA for BXD strains revealed a main effect of strain [$F(20, 240)=5.0$, $p<0.001$] and a strain by PNS interaction [$F(20, 240)=1.7$, $p=0.03$] (see figure 2b). The heritability for control condition is 0.2 and for PNS is 0.27.

The intra-class correlation for assessment of reliability was ICC $(1, 2) = 0.98$, 0.92 and 1.0 for the 930, 1300 and 1700 observation session, respectively. These results indicate high inter-observer reliability.

The association between strain-level, stress-induced CORT change and strain-level, stress-induced dam-pup contact was assessed. A positive, significant correlation was detected ($r=0.51$, $p=0.01$) (see figure 2c).

Both the strain CORT responses and maternal behavior data were uploaded to genenetwork,org and are publicly available (genenetwork IDs: baseline CORT 19509, CORT stress response 19508, maternal behavior stress response (PNS minus control mean) 18651, maternal behavior control group 21454, maternal behavior PNS group 21455).

Associations of Maternal Stress Response to Offspring Phenotype

The strain CORT response scores (difference from baseline to post-stress) were assessed for correlations to strain difference scores for acute locomotion/sensitization and CPP. For males, a significant, positive correlation was detected for acute locomotion ($r=0.48$, $p=0.03$) (see figure 3a) and CPP ($r=0.43$, $p=0.04$) (see figure 3b) difference scores. No other significant correlations were detected.

The strain difference scores for maternal-pup contact were assessed for correlations to strain difference scores for acute locomotion/sensitization and CPP. A significant, positive correlation was found between maternal-pup contact and CPP difference scores in males ($r=0.57$, $p<0.01$) (see figure 3c). No other correlations were detected. See table 1 for offspring mean/SEM/sample size within strain/condition/sex for cocaine-phenotypes.

Re-mapping QTLs with Adjusted Means

The estimated marginal means (EMMs) for acute locomotion and CPP in PNS subjects were calculated with maternal CORT response as a covariate. EMMs were also calculated for CPP with the dam-pup contact scores as a covariate for both control and PNS subjects. The difference scores for these means were determined and then subjected to QTL mapping. A significant QTL on chromosome X (LRS=16.6, peak marker=rs13483729, location 38.899709) was detected for acute cocaine locomotion with CORT adjusted means in the same location as the QTL detected for unadjusted means (Bagley et. al., 2019). No other significant QTLs were detected.

In order to assess the possibility that the effects of the acute cocaine locomotion QTL are mediated by effects on maternal CORT or maternal-pup contact, we grouped all BXD strains by their genotype at this locus (B6 or D2). A t-test was performed between the two genotype groups for strain maternal CORT response and dam-pup contact strain differences scores (CORT mean ± SEM, B6 allele= 1244.5 ± 191.4, D2 allele= 1623.6 ± 423.7; maternal-pup contact mean ± SEM, B6 allele= −1.8 ± 1.4, D2 allele= 1.6 ± 2.4). No significant effects were detected for CORT [t(19)=0.9, p=0.688] or maternal-pup contact [t(19)=1.2, p=0.999].

Discussion

The present experiment indicates a gene-dependent impact of stress during gestation on both neuroendocrine response at the time of stress as well as subsequent maternal-pup interactions during the early postnatal period. We observed the CORT response to restraint-stress to be a heritable trait, with strain differences in the magnitude of the CORT response. Using a non-invasive examination, we observed that repeated restraint stress during the last week of gestation interacts with strain to alter maternal-pup contact in the postnatal period, indicating this effect of PNS to also be heritable in the BXD panel. These data collectively reveal BXD strain differences in the dam response to restraint stress that may have consequences for prenatal and postnatal development of the offspring. The strain effects of restraint stress on dam-pup contact associated with the strain-dependent effects of PNS on offspring cocaine-induced CPP. Additionally, the strain-dependent maternal CORT response associated with the strain-dependent effects of PNS on cocaine-induced locomotion and CPP. These findings demonstrate that strain-dependent, multimodal aspects of the maternal stress response may mediate the impact of PNS on the offspring cocaine phenotypes.

Pregnant dams of the B6 and D2 strains demonstrated reliable restraint-induced elevations in CORT across the PNS procedure period indicating that the restraint procedure is an effective stressor in these strains. The magnitude of the CORT response differed between strains and was consistent over both stress sessions tested indicating that this testing is sufficient to detect stable strain differences. Further, neither of the strains exhibited habituation of the CORT response to restraint stress indicating that, in pregnant females, restraint stress is a reliable neuroendocrine challenge over multiple exposures. However, the baseline CORT levels increased from day 1 to day 5 in both strains which is consistent with previously reported CORT elevations throughout pregnancy, including a sharp increase shortly before parturition [40,41]. Interestingly, the magnitude of this shift was greater in B6 mouse, indicating heritability of this trait, which may have strain-dependent consequences for glucocorticoid programming of developing offspring even in the absence of overt, procedural stressor exposure. However, the relation of baseline CORT measures to developmental outcomes is beyond the scope of the present report and was not evaluated in the BXD strains.

BXD strains exhibited marked variation in restraint stress-induced elevations during pregnancy that exceeded the range demarcated by the progenitor strains and moreover, these changes predicted subsequent behavioral alterations produced by PNS. Associations between maternal CORT response and cocaine locomotion/CPP suggest that the effects of stress-induced CORT on these phenotypes may be plasma concentration dependent. Some

effects of PNS are known to be dependent on stress-induced surges in CORT levels which have been mimicked by exogenous administration of CORT or synthetic glucocorticoids [42,43]. Most studies utilize a single dose in an effort to simulate endogenous surges in stress-induced CORT. For instance, in a study by Weinstock and colleagues [16], the average elevation of CORT in pregnant rats exposed to stress was determined and CORT dosing procedures, to accurately reproduce the profile of the maternal average, were developed and shown to impact offspring behavior. Although this study makes the point that dose is extremely important, it fails to recapitulate variation in endogenous stress response as a potential critical mediator of individual vulnerability to PNS. Another study examining PNS in transgenic mice found gene-dependent maternal CORT responses, with CRFR2 knock-out mice being more sensitive to stressors and exhibiting greater stress-induced elevations in CORT compared to WT. The offspring of the PNS KO mice had lower body weights relative to PNS WT mice, and this effect persisted into adulthood, suggesting the genetically-determined magnitude of the stress response may have affected offspring development [44]. This evidence indicates maternal stress sensitivity may have an enduring impact on the phenotype of PNS offspring. Furthermore, a dose-response relationship with the synthetic glucocorticoid dexamethasone was detected for hippocampal degeneration in rats, with greater degeneration occurring with greater dose [45]. Similarly, reduction in fetal growth by dexamethasone administration to rats was found to be dose-dependent [45]. These studies indicate that the dose-response of deleterious glucocorticoid effects can be graded, with a threshold dose for harmful effects, and increasing severity with increasing dose. A similar dose-response relationship of endogenous CORT may cause differential consequences for fetal development and indicate the importance of the maternal stress CORT response variability. Nevertheless, despite the compelling data implicating glucocorticoids in fetal programming and PNS effects, potential implications of CORT dose-dependent effects for gene/strain-dependent maternal responsiveness to stress during in-utero programing, subsequent maternal behavior perturbations, or their potential compounding impacts on neonatal development of the offspring remain unknown.

The effect of PNS on dam-pup contact was also found to associate with the effect of PNS on CPP. These results suggest that genetically moderated changes in maternal behavior may also be a mechanism by which PNS differentially alters cocaine responsiveness in BXD strains. Variation in maternal behavior is known to be a determinant of physiological and behavioral outcomes in the offspring, with associations to HPA axis function being most commonly presented [46]. The effects of maternal care are also implicated in cocaine responsiveness, as maternal licking/grooming during early life is reported to negatively associate with cocaine self-administration in adulthood [32]. Furthermore, reductions in litter size, a manipulation that increases licking/grooming, decreases cocaine locomotor sensitization and CPP in males but not females [33]. The male-specific sensitivity to the effects of PNS on cocaine CPP is congruent with male-specific effects of PNS on cocaine CPP/locomotor behaviors in BXD strains, reported in Bagley et. al. 2019 and with the male-specific correlations to maternal factors in the present study. However, here we observed that reduced dam-pup contact was associated with decreased CPP which appears in apparent opposition to other studies. However, we did not measure licking/grooming. Dam-pup contact and licking/grooming are not necessarily associated; in one instance a prenatal

manipulation increased licking/grooming with no change in total contact and natural variations in rat licking/grooming do not associate with total contact [47,48]. Thus, a more detailed analysis of dam-pup interactions across strains may illuminate further relations between PNS-induced changes in early life that impact later drug addiction vulnerability. Furthermore, offspring responses to cocaine were tested at a single dose in the BXD strains. This limits the ability to discern effects on cocaine sensitivity from effects on cocaine efficacy. Although previous study of the BXD progenitor strains at multiple cocaine doses indicated PNS caused a strain-biased upward shift in the CPP dose response curve[7], this does not precluded the possibility that leftward/rightward shifts may occur across BXD strains. Collectively, contributions of heritable differences in maternal stress response, and the nature of PNS- induced changes to cocaine locomotion/reward in the offspring, may be best explored once candidate genes are selected for direct manipulation and study. At this level of analysis, expansive phenotyping becomes feasible and the gene's role and mechanism of action can be better elucidated.

The results of the present experiment indicate a PNS-independent strain effect on maternal behavior in the progenitor and BXD strains. D2 mice were found to have more contact with their pups than B6 mice. A similar strain effect was also identified by a study in which B6 and D2 maternal behavior were compared. D2 mice were found to score higher on multiple measures of maternal behavior including nursing, nest building and contact rest [49]. These similarities may indicate congruence and replicability of the dam-pup interaction traits measured across this study and the present study. No effects of PNS were found on progenitor dam-pup contact in the present study, despite strain by PNS interactions in the BXD strains. Although differences between the progenitor strains indicate heritability in the BXD panel, in some cases genetic effects are only present in the BXD strains, due to unique allele combinations in BXD strains that allow for the emergence of strain differences.

Intriguingly, there was a significant correlation of the maternal neuroendocrine response to stress and subsequent maternal behavior suggesting a bidirectional effect. Importantly, all strains exhibited an elevation in CORT following restraint stress, however, somewhat unexpectedly, dam-pup contact reductions were associated with lower stress-induced elevations in CORT whereas strains exhibiting higher stress-induced elevations in CORT exhibited increased dam-pup contacts. Although interpretation of these relations require caution both because of the correlational bases and the somewhat cursory measures (i.e. dam-pup contact vs detailed quantitative analyses of multiple maternal behaviors, single CORT measurement at end of stress vs full-time course of CORT response, etc.), they suggest that neuroendocrine stress response magnitude is an important mediator of subsequent stress-induced perturbations.

Although maternal behavior is an important driver of pup development, pup physiology and behavior are known to be determinants of maternal behavior. This is demonstrated by a study in which BXD pups were cross-fostered to B6 dams. Alterations in the B6 dam maternal behaviors are dependent on the strain of the pups [50]. PNS induced changes in rat pups also alter maternal behavior. Stressed pups under care of unstressed dams elicit less licking/grooming, contact time and retrieval rates relative to controls pups [28,51,52]. This effect may be specific to male pups and mediated by changes in urine composition, as urine

of stressed male pups elicited less investigation by dams relative to controls [28]. Considering that PNS induced changes in pup physiology and behavior may alter maternal behavior, such changes in BXD strains may fully or partially explain the association between PNS alterations in maternal behavior and PNS changes in CPP in the present study. Pups of strains more affected by PNS exposure may cause larger shifts in the behavior for their dams. In this case, these maternal care changes may not play an entirely independent, causal role in subsequent adult cocaine phenotypes of the offspring but could be reflecting genetically determined PNS effects on the offspring. Adjustment of offspring strain means for cocaine CPP/locomotion by both maternal contact and CORT covariates yielded the same PNS interacting QTL as unadjusted means. These results suggest that this QTL is not reflecting strain variance imparted by heritable maternal response, and may reflect a gene that interacts with PNS exposure to modulate offspring development. Collectivley, these results highlight the complexity of PNS effects and interactions with genetics that may be dissected by assessment of appropriate traits.

Summary

The developmental effects of PNS are mediated by the maternal stress response, with physiological and behavioral stress reactions of the mother as potential modes of transmission to the offspring. It is likely that differential maternal stress sensitivity modulates the impact of PNS on offspring development. Genetic variants may be a source of differences in the maternal stress response, including BXD strain differences. Thus, in considering heritable differences in the offspring response to PNS, the heritable differences in the maternal stress response must be considered as a source of variation. Furthermore, heritable differences in the maternal stress response may yield a rich source of data that reveals genes that moderate the effects of stress on the in-utero environment and maternal care. Given that in-utero conditions and early life maternal care may have profound effects on subsequent drug abuse risk, among many other psychiatric disorders [2], understanding the stress by gene interactions that moderate maternal HPA activity and maternal care may be of great value in identifying novel genetic contributors to disease states as well as delineating mechanisms underlying differences in vulnerability and resilience to disease.

Acknowledgements

References

1. McGinnis JM & Foege WH Mortality and Morbidity Attributable to Use of Addictive Substances in the United States. Proceedings of the Association of American Physicians 111, 109–118 (1999). [PubMed: 10220805]

2. Enoch M-A The Role of Early Life Stress as a Predictor for Alcohol and Drug Dependence. Psychopharmacology (Berl) 214, 17–31 (2011). [PubMed: 20596857]

3. Kippin TE, Szumlinski KK, Kapasova Z, Rezner B & See RE Prenatal Stress Enhances Responsiveness to Cocaine. Neuropsychopharmacology 33, 769–782 (2008). [PubMed: 17487224]

4. Pastor V, Pallarés ME & Antonelli MC Prenatal stress increases adult vulnerability to cocaine reward without affecting pubertal anxiety or novelty response. Behavioural Brain Research 339, 186–194 (2018). [PubMed: 29191579]

5. Thomas MB & Becker JB Sex differences in prenatal stress effects on cocaine pursuit in rats. Physiol. Behav (2017). doi:10.1016/j.physbeh.2017.10.019

6. Thomas MB, Hu M, Lee TM, Bhatnagar S & Becker JB Sex-specific susceptibility to cocaine in rats with a history of prenatal stress. Physiol. Behav 97, 270–277 (2009). [PubMed: 19268677]

7. Kippin TE, Campbell JC, Ploense K, Knight CP & Bagley J Prenatal stress and adult drug-seeking behavior: interactions with genes and relation to nondrug-related behavior. Adv Neurobiol 10, 75–100 (2015). [PubMed: 25287537]

8. Bagley JR, Szumlinski KK & Kippin TE Discovery of early life stress interacting and sex-specific quantitative trait loci impacting cocaine responsiveness. British Journal of Pharmacology (2019).

9. Herman JP & Cullinan WE Neurocircuitry of stress: central control of the hypothalamo–pituitary–adrenocortical axis. Trends in Neurosciences 20, 78–84 (1997). [PubMed: 9023876]

10. Barbazanges A, Piazza PV, Le Moal M & Maccari S Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. J. Neurosci 16, 3943–3949 (1996). [PubMed: 8656288]

11. Montano MM, Wang MH, Even MD & vom Saal FS Serum corticosterone in fetal mice: sex differences, circadian changes, and effect of maternal stress. Physiol. Behav 50, 323–329 (1991). [PubMed: 1745676]

12. Mueller BR & Bale TL Early prenatal stress impact on coping strategies and learning performance is sex dependent. Physiol. Behav 91, 55–65 (2007). [PubMed: 17367828]

13. Takahashi LK Prenatal stress: consequences of glucocorticoids on hippocampal development and function. Int. J. Dev. Neurosci 16, 199–207 (1998). [PubMed: 9785116]

14. Zagron G & Weinstock M Maternal adrenal hormone secretion mediates behavioural alterations induced by prenatal stress in male and female rats. Behavioural Brain Research 175, 323–328 (2006). [PubMed: 17023059]

15. Diaz R, Ogren SO, Blum M & Fuxe K Prenatal corticosterone increases spontaneous and d-amphetamine induced locomotor activity and brain dopamine metabolism in prepubertal male and female rats. Neuroscience 66, 467–473 (1995). [PubMed: 7477887]

16. Salomon S, Bejar C, Schorer-Apelbaum D & Weinstock M Corticosterone Mediates Some but Not Other Behavioural Changes Induced by Prenatal Stress in Rats. Journal of Neuroendocrinology 23, 118–128 (2011). [PubMed: 21108672]

17. Rodrigues AJ et al. Mechanisms of initiation and reversal of drug-seeking behavior induced by prenatal exposure to glucocorticoids. Mol Psychiatry 17, 1295–1305 (2012). [PubMed: 21968930]

18. Doering CH, Shire JG, Kessler S & Clayton RB Cholesterol ester concentration and corticosterone production in adrenals of the C57BL-10 and DBA-2 strains in relation to adrenal lipid depletion. Endocrinology 90, 93–101 (1972). [PubMed: 4333015]

19. Freund RK, Martin BJ, Jungschaffer DA, Ullman EA & Collins AC Genetic differences in plasma corticosterone levels in response to nicotine injection. Pharmacol. Biochem. Behav 30, 1059–1064 (1988). [PubMed: 3227029]

20. Roberts AJ, Phillips TJ, Belknap JK, Finn DA & Keith LD Genetic analysis of the corticosterone response to ethanol in BXD recombinant inbred mice. Behav. Neurosci 109, 1199–1208 (1995). [PubMed: 8748968]

21. Golub Y et al. Effects of In utero environment and maternal behavior on neuroendocrine and behavioral alterations in a mouse model of prenatal trauma: Mouse Model of Prenatal Trauma. Developmental Neurobiology 76, 1254–1265 (2016). [PubMed: 26899175]

22. Kinsley C & Svare B Prenatal stress alters maternal aggression in mice. Physiology & Behavior 42, 7–13 (1988). [PubMed: 3387480]

23. Meek LR, Dittel PL, Sheehan MC, Chan JY & Kjolhaug SR Effects of stress during pregnancy on maternal behavior in mice. Physiology & Behavior 72, 473–479 (2001). [PubMed: 11282130]

24. Pardon M, Gérardin P, Joubert C, Pérez-Diaz F & Cohen-Salmon C Influence of prepartum chronic ultramild stress on maternal pup care behavior in mice. Biol. Psychiatry 47, 858–863 (2000). [PubMed: 10807958]

25. Baker S et al. Effects of gestational stress: 1. Evaluation of maternal and juvenile offspring behavior. Brain Research 1213, 98–110 (2008). [PubMed: 18456246]

26. Champagne FA & Meaney MJ Stress During Gestation Alters Postpartum Maternal Care and the Development of the Offspring in a Rodent Model. Biological Psychiatry 59, 1227–1235 (2006). [PubMed: 16457784]

27. Patin V et al. Effects of prenatal stress on maternal behavior in the rat. Developmental Brain Research 139, 1–8 (2002). [PubMed: 12414088]

28. Power KL & Moore CL Prenatal stress eliminates differential maternal attention to male offspring in Norway rats. Physiology & Behavior 38, 667–671 (1986). [PubMed: 3823181]

29. Smith JW, Seckl JR, Evans AT, Costall B & Smythe JW Gestational stress induces post-partum depression-like behaviour and alters maternal care in rats. Psychoneuroendocrinology 29, 227–244 (2004). [PubMed: 14604603]

30. Francis DD, Diorio J, Liu D & Meaney MJ Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science 286, 1155–1158 (1999). [PubMed: 10550053]

31. Liu D et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 277, 1659–1662 (1997). [PubMed: 9287218]

32. Francis DD & Kuhar MJ Frequency of maternal licking and grooming correlates negatively with vulnerability to cocaine and alcohol use in rats. Pharmacol. Biochem. Behav 90, 497–500 (2008). [PubMed: 18508115]

33. Li Y-Q et al. Effects of early postnatal sibling deprivation on anxiety and vulnerability to cocaine in offspring rats. Psychopharmacology (Berl.) 199, 245–253 (2008). [PubMed: 18452033]

34. Moffett MC et al. Maternal separation and handling affects cocaine self-administration in both the treated pups as adults and the dams. J. Pharmacol. Exp. Ther 317, 1210–1218 (2006). [PubMed: 16517692]

35. Camp MC et al. Genetic Strain Differences in Learned Fear Inhibition Associated with Variation in Neuroendocrine, Autonomic, and Amygdala Dendritic Phenotypes. Neuropsychopharmacology 37, 1534–1547 (2012). [PubMed: 22334122]

36. Gibb J, Hayley S, Poulter MO & Anisman H Effects of stressors and immune activating agents on peripheral and central cytokines in mouse strains that differ in stressor responsivity. Brain, Behavior, and Immunity 25, 468–482 (2011).

37. Priebe K et al. Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/cJ mice: A cross-fostering study. Developmental Psychobiology 47, 398–407 (2005). [PubMed: 16284964]

38. Shanks N, Griffiths J, Zalcman S, Zacharko RM & Anisman H Mouse strain differences in plasma corticosterone following uncontrollable footshock. Pharmacology Biochemistry and Behavior 36, 515–519 (1990).

39. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals (National Academies Press (US), 2011).

40. Barlow SM, Morrison PJ & Sullivan FM Plasma corticosterone levels during pregnancy in the mouse: the relative contributions of the adrenal glands and foeto-placental units. J. Endocrinol 60, 473–483 (1974). [PubMed: 4823252]

41. Montano MM, Wang MH, Even MD & vom Saal FS Serum corticosterone in fetal mice: sex differences, circadian changes, and effect of maternal stress. Physiol. Behav 50, 323–329 (1991). [PubMed: 1745676]

42. Harris A & Seckl J Glucocorticoids, prenatal stress and the programming of disease. Horm Behav 59, 279–289 (2011). [PubMed: 20591431]

43. Seckl. Prenatal glucocorticoids and long-term programming. European Journal of Endocrinology 151, U49–U62 (2004). [PubMed: 15554887]

44. Mueller BR & Bale TL Impact of prenatal stress on long term body weight is dependent on timing and maternal sensitivity. Physiol. Behav 88, 605–614 (2006). [PubMed: 16828814]

45. LaBorde JB, Hansen DK, Young JF, Sheehan DM & Holson RR Prenatal dexamethasone exposure in rats: effects of dose, age at exposure, and drug-induced hypophagia on malformations and fetal organ weights. Fundam Appl Toxicol 19, 545–554 (1992). [PubMed: 1426713]

46. Champagne FA Maternal imprints and the origins of variation. Horm Behav 60, 4–11 (2011). [PubMed: 21376726]

47. Menard JL & Hakvoort RM Variations of maternal care alter offspring levels of behavioural defensiveness in adulthood: evidence for a threshold model. Behav. Brain Res 176, 302–313 (2007). [PubMed: 17084914]

48. Palanza PL, Howdeshell KL, Parmigiani S & vom Saal FS Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. Environ Health Perspect 110, 415–422 (2002). [PubMed: 12060838]

49. Shoji H & Kato K Maternal behavior of primiparous females in inbred strains of mice: a detailed descriptive analysis. Physiol. Behav 89, 320–328 (2006). [PubMed: 16842828]

50. Ashbrook DG, Gini B & Hager R Genetic variation in offspring indirectly influences the quality of maternal behaviour in mice. Elife 4, (2015).

51. Moore CL & Power KL Prenatal stress affects mother-infant interaction in Norway rats. Dev Psychobiol 19, 235–245 (1986). [PubMed: 3709978]

52. Pérez-Laso C et al. Maternal care interacts with prenatal stress in altering sexual dimorphism in male rats. Horm Behav 64, 624–633 (2013). [PubMed: 23994571]

Highlights

- The maternal HPA axis response to gestational stress is heritable in the BXD recombinant inbred strain panel

- Prenatal stress alters dam-pup contact in a strain dependent manner

- Heritable variation in the maternal stress response associates with heritable variation of prenatal stress effects on offspring responses to cocaine
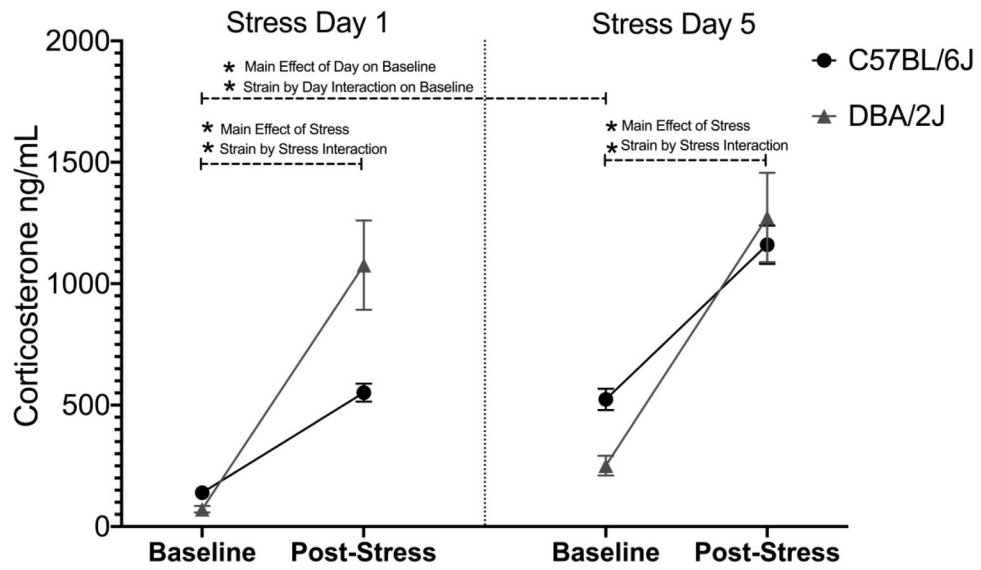
Figure 1.
Effects of restraint stress on plasma corticosterone in pregnant BXD progenitor strains, on stress day 1 and 5 (mean and SEM). Both strains demonstrated increased CORT as a result of restraint, but DBA/2J mice had a greater increase on both days. Baseline CORT levels increased from day 1 to 5 in both strains, but C57BL/6J mice had a greater increase.
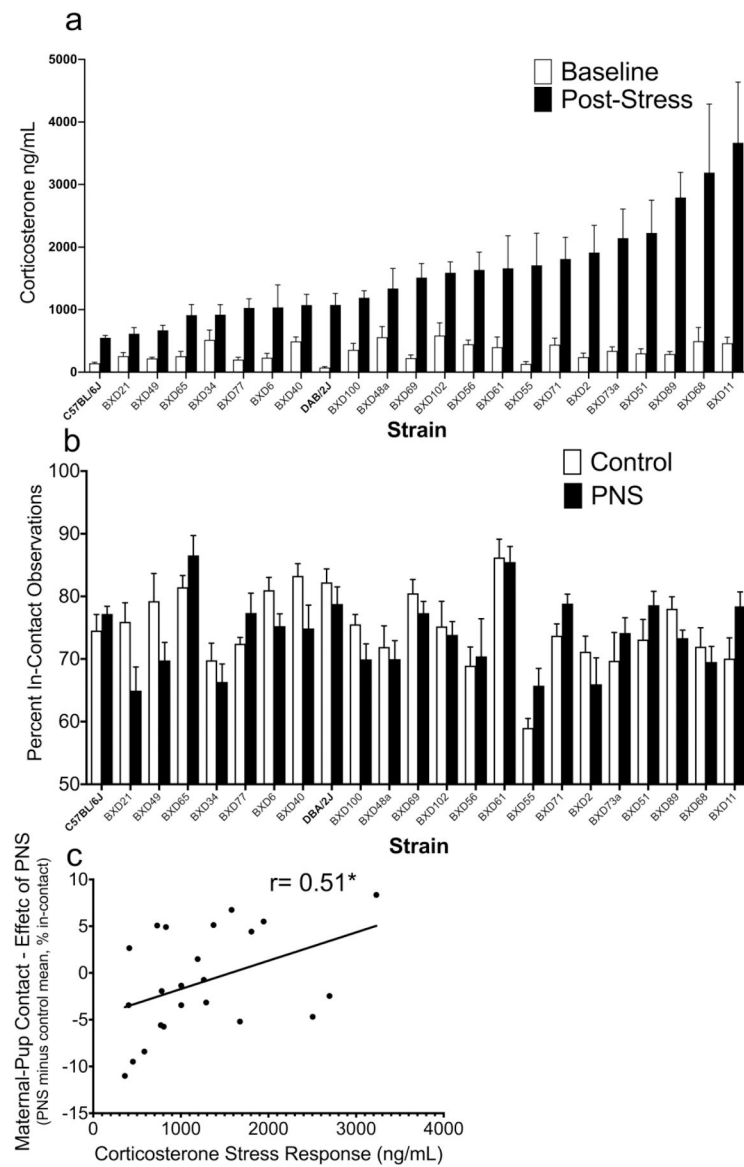
Figure 2.
Effects of restraint stress on maternal corticosterone and dam-pup contact (mean and SEM, progenitor strains are labeled in bold). a) Strain affects the degree of the CORT response to restraint stress (strain by restraint stress interaction, $p < 0.05$). b) Shifts in dam-pup contact are affected by strain (strain by restraint stress interaction, $p < 0.05$). c) A significant correlation ($p < 0.05$) between the strain level CORT response to restraint stress and PNS induced, strain level changes in dam-pup contact.
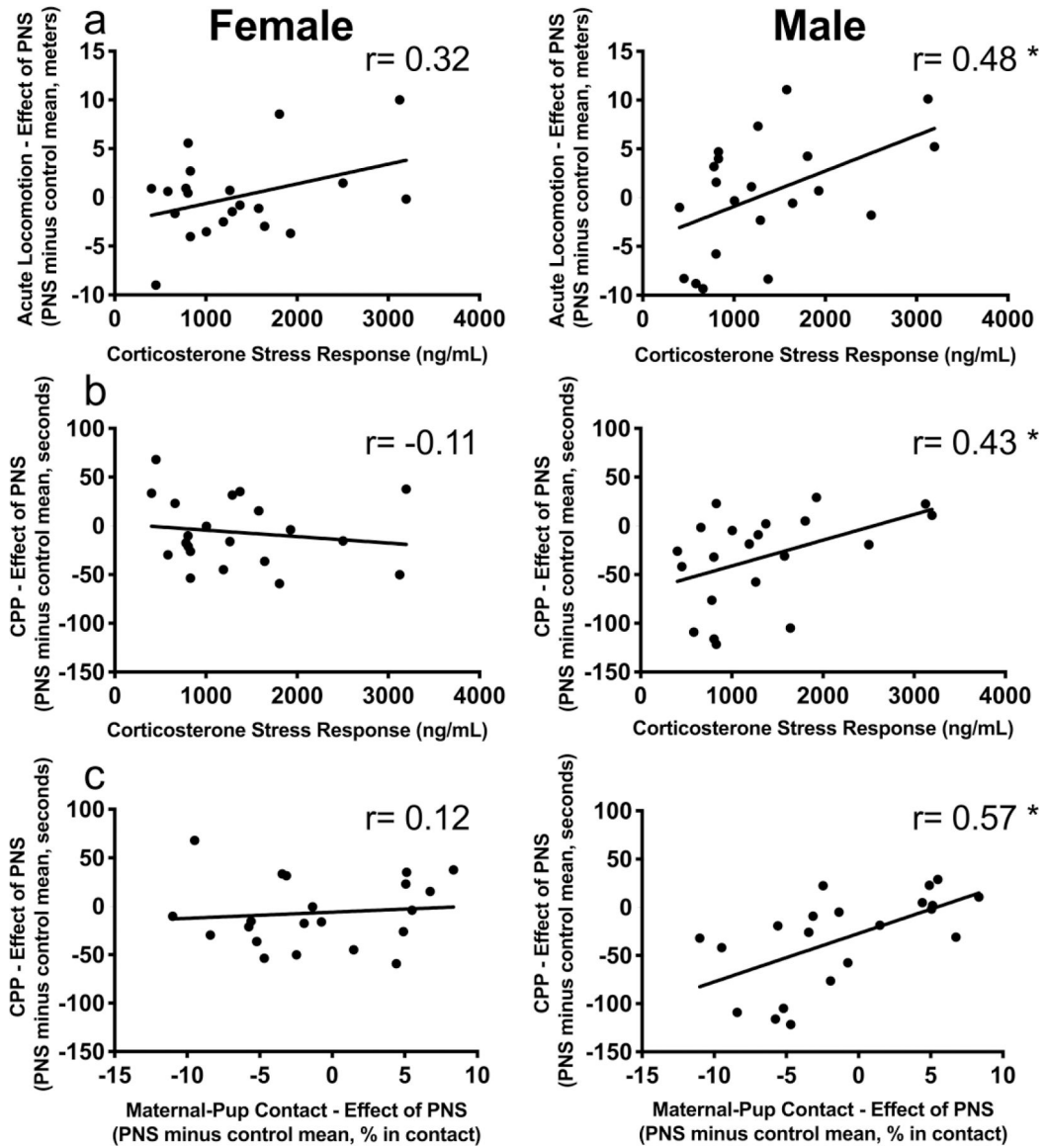
Figure 3.
Associations between strain maternal stress response and strain PNS effects on offspring cocaine phenotypes (data points represent PNS minus control strain mean for acute locomotion, CPP and maternal contact. The CORT data points represent the strain level CORT response to stress (baseline to post-stress change). a) Significant correlation between maternal CORT response and male acute locomotion difference scores. b) Significant correlation between maternal CORT response and male CPP difference scores. c) Significant correlation between maternal-pup contact difference scores and male CPP.

Table 1

Strain X Condition X Sex data for acute locomotor response, locomotor sensitization and conditioned place preference response to cocaine. Data presented as mean ± SEM (sample size).

Strain	Acute Locomotor Response (meters)				Locomotor Sensitization (meters)				Conditioned Place Preference (seconds)			
	Control		PNS		Control		PNS		Control		PNS	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
BXD2	30±2 (12)	27±4 (17)	27±2 (12)	26±2 (12)	5±3 (13)	1±3 (18)	3±4 (12)	-2±4 (12)	170±40 (13)	192±27 (18)	133±29 (13)	87±44 (12)
BXD6	25±5 (10)	19±4 (14)	30±5 (14)	21±5 (12)	5±5 (10)	11±4 (14)	15±5 (13)	20±4 (11)	185±28 (9)	151±26 (14)	163±36 (14)	35±46 (12)
BXD11	18±4 (13)	13±2 (11)	17±4 (12)	18±3 (8)	24±4 (14)	27±5 (12)	15±4 (10)	21±8 (8)	270±59 (13)	233±56 (12)	308±41 (12)	243±51 (9)
BXD21	35± 3 (10)	28±3 (17)	36±3 (13)	22±4 (13)	10±3 (10)	24±3 (16)	10±4 (13)	22±5 (13)	141±23 (10)	131±22 (16)	131±18 (13)	99±25 (13)
BXD34	12±2 (13)	14±3 (12)	13±2 (13)	13±2 (10)	21±2 (14)	16±3 (11)	18±3 (12)	15±4 (9)	183±29 (13)	208±21 (12)	217±27 (11)	183±40 (9)
BXD40	29±2 (12)	40±3 (12)	29±3 (15)	31±3 (12)	24±5 (15)	28±4 (13)	20±3 (14)	19±4 (12)	217±22 (14)	234±14 (12)	187±36 (16)	125±24 (13)
BXD48a	16±2 (13)	15±3 (14)	17±2 (12)	18±2 (12)	21±3 (13)	16±3 (16)	18±5 (12)	16±4 (12)	152±21 (13)	196±29 (16)	134±30 (12)	120±38 (12)
BXD49	26±4 (14)	32±3 (10)	17±3 (12)	24±6 (10)	5±3 (14)	-1±3 (10)	-1±5 (12)	3±5 (10)	101±17 (14)	143±24 (11)	169±24 (11)	101±31 (11)
BXD51	16±2 (10)	11±1 (13)	13±2 (10)	12±2 (12)	9±3 (10)	13±2 (13)	16±2 (10)	11±2 (11)	166±22 (9)	128±22 (14)	162±27 (10)	157±20 (11)
BXD55	15±3 (12)	9±3 (12)	14±2 (15)	20±2 (14)	1±2 (11)	16±4 (12)	6±3 (15)	5±3 (16)	101±12 (12)	122±17 (12)	116±19 (16)	91±15 (15)
BXD56	13±4 (13)	9±4 (14)	10±3 (11)	10±3 (10)	6±4 (11)	26±5 (14)	3±6 (11)	20±7 (10)	290±26 (11)	362±25 (13)	245±39 (11)	343±49 (10)
BXD61	20±3 (9)	32±6 (10)	20±4 (11)	39±12 (8)	14±2 (9)	11±4 (10)	12±3 (11)	5±9 (7)	140±25 (9)	185±29 (11)	124±28 (13)	127±35 (8)
BXD65	19±3 (17)	23±2 (16)	17±3 (14)	14±2 (9)	10±2 (16)	12±3 (16)	9±2 (13)	9±4 (9)	133±18 (18)	136±14 (17)	156±14 (14)	134±26 (10)
BXD68	17±3 (9)	15±3 (10)	27±3 (14)	25±3 (13)	15±4 (10)	20±2 (10)	11±5 (13)	11±3 (13)	201±31 (10)	79±35 (10)	151±22 (13)	102±27 (13)
BXD69	29±2 (10)	25±5 (12)	27±2 (16)	22±3 (13)	9±2 (11)	21±9 (12)	13±4 (15)	27±5 (12)	103±19 (12)	143±24 (11)	134±24 (16)	134±29 (13)
BXD71	27±2 (8)	21±2 (9)	26±3 (9)	12±2 (11)	9±5 (7)	23±3 (9)	13±3 (9)	22±2 (10)	62±19 (8)	167±25 (10)	97±15 (9)	169±25 (11)
BXD73a	38±3 (12)	38±4 (18)	46±3 (16)	43±4 (12)	2±5 (14)	22±6 (17)	7±3 (16)	5±3 (14)	157±27 (14)	153±22 (18)	98±18 (16)	158±21 (13)
BXD77	27±3 (14)	27±4 (14)	29±3 (17)	31±3 (15)	6±5 (14)	10±6 (13)	15±3 (17)	11±4 (14)	176±35 (15)	142±22 (14)	150±18 (19)	165±17 (15)
BXD89	15±2 (13)	18±3 (13)	17±2 (15)	16±3 (13)	1±5 (14)	5±3 (12)	3±2 (14)	8±4 (13)	86±26 (14)	130±31 (13)	71±18 (14)	111±27 (13)
BXD100	11±1 (17)	8±1 (14)	7±2 (17)	13±3 (12)	16±2 (18)	11±3 (14)	9±2 (17)	4±3 (12)	240±23 (18)	286±19 (14)	187±19 (17)	164±33 (12)
BXD102	23±2 (10)	25±3 (15)	20±2 (14)	24±2 (9)	19±5 (10)	19±4 (15)	25±3 (15)	16±4 (10)	148±29 (11)	135±23 (14)	147±18 (16)	130±23 (10)