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Exposure to prenatal life events stress is associated with masculinized play behavior in girls

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Abstract

Previous research has shown that prenatal exposure to endocrine-disrupting chemicals can alter children's neurodevelopment, including sex-typed behavior, and that it can do so in different ways in males and females. Non-chemical exposures, including psychosocial stress, may disrupt the prenatal hormonal milieu as well. To date, only one published study has prospectively examined the relationship between exposure to prenatal stress and gender-specific play behavior during childhood, finding masculinized play behavior in girls who experienced high prenatal life events stress, but no associations in boys. Here we examine this question in a second prospective cohort from the Study for Future Families. Pregnant women completed questionnaires on stressful life events during pregnancy, and those who reported one or more events were considered "stressed". Families were recontacted several years later (mean age of index child: 4.9 years), and mothers completed a questionnaire including the validated Preschool Activities Inventory (PSAI), which measures sexually dimorphic play behavior. In sex-stratified analyses, after adjusting for child's age, parental attitudes towards gender-atypical play, age and sex of siblings, and other relevant covariates, girls (n=72) exposed to prenatal life events stress had higher scores on the PSAI masculine sub-scale (β=3.48, p=0.006) and showed a trend towards higher (more masculine) composite scores (β=2.63, p=0.08). By contrast, in males (n=74), there was a trend towards an association between prenatal stress and higher PSAI feminine sub-scale scores (β=2.23, p=0.10), but no association with masculine or composite scores. These data confirm previous findings in humans and animal models suggesting that prenatal stress is a non-chemical endocrine disruptor that may have androgenic effects on female fetuses and anti-androgenic effects on male fetuses.

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Keywords

prenatal stress; stress; pregnancy; play behavior; PSAI; sex differences; androgens; testosterone

Introduction

In utero sexual differentiation of the mammalian brain is driven by gonadal steroid production. Androgens produced by male fetuses starting in the late first trimester are aromatized to estradiol, which, in turn, masculinizes the brain. In the absence of androgens, conversely, the brain develops in a feminine manner. Not surprisingly, therefore, endocrine-disrupting chemicals that alter androgen production or activity appear to affect both reproductive and brain development. Rodents exposed to anti-androgenic chemicals including phthalates, vinclozolin, and flutamide show reduced male-typical play behavior as well as incomplete masculinization of the genitals (Casto et al., 2003, Colbert et al., 2005, Hotchkiss et al., 2003). Similar associations are seen in humans: males exposed to higher levels of phthalates *in utero* have shorter anogenital distance (AGD), an indicator of prenatal androgen exposure, in infancy and reduced masculine play behavior in childhood (Swan et al., 2010, Swan et al., 2005). Interestingly, in animal models, the effects of prenatal exposure to endocrine-disrupting chemicals are mirrored by the effects of prenatal exposure to stress. Male rodents born to stressed dams have lower testes weight and shorter anogenital distance (AGD), an indicator of decreased prenatal androgen exposure, (Dahlof et al., 1978) and play in a more feminine manner compared to controls (Ward and Stehm, 1991). In adulthood, their testosterone levels tend to be lower, they display altered mating behavior, and are less fertile than controls (Crump and Chevins, 1989, Kemme et al., 2007, Ward, 1972).

Interestingly, in these animal models, prenatal stress also affects female offspring, but in the opposite direction. Whereas males show evidence of reduced androgens following prenatal stress, females tend to show more masculine AGD, higher testosterone, and more masculine courtship, play, and social behavior than controls (Kaiser and Sachser, 1998, Kinsley and Bridges, 1988, vom Saal et al., 1990), as well as problems conceiving and maintaining a pregnancy (Herrenkohl, 1979). In humans, we recently showed that prenatal life events stress is associated with longer, more masculine AGD in female infants as well as a trend towards shorter, less masculine AGD in male infants (Barrett et al., 2013). This pattern of results suggests that prenatal stress may act as a non-chemical endocrine disruptor, interfering with sex-typical reproductive and neurodevelopment. That the hypothalamic-pituitary-adrenal (HPA) stress axis and in particular, cortisol, can affect the hypothalamic-pituitary-gonadal (HPG) reproductive axis is well known (Lovejoy and Barsyte-Lovejoy, 2013, Viau, 2002), and it is possible that these effects may occur across the maternal-fetal-placental unit as well.

If prenatal stress alters the sex-typical *in utero* hormonal milieu, then it is likely to have effects on neurodevelopment as well, particularly in domains that are both sexually dimorphic and plausibly androgen-related, such as play behavior. During childhood, there are strong sex differences in play behavior, and these differences are robust across many

play behavior assessments and experimental paradigms (Berenbaum and Hines, 1992). Sex differences in play behavior emerge very early in development. As early as 3–8 months of age, male and female infants show differences in visual attention to dolls and trucks (Alexander et al., 2009). Sex-specific play behavior is also evident in other mammalian species as well. Male rhesus macaques and vervet monkeys show preferences for masculine play (such as playing with wheeled toys), while females show preferences, though less consistent, for playing with feminine toys (such as dolls) (Alexander and Hines, 2002, Hassett et al., 2008). Even male and female rodents "play" differently, with males tending to engage in more rough-and-tumble play (Pellis, 2002). Pre- and early postnatal androgens appear to play an important role in the development of these sex differences in play behavior (Collaer and Hines, 1995). Testosterone levels in both maternal serum and amniotic fluid have been positively associated with more masculine Preschool Activities Inventory (PSAI) scores in girls and boys (amniotic fluid only), in some, but not all, studies (Auyeung et al., 2009, Hines et al., 2002a, Knickmeyer et al., 2005, van de Beek et al., 2009), and urinary testosterone during the infant male "mini puberty" (believed to occur in early infancy, approximately age 3–4 months) has been linked to toy preferences and play behavior at 14 months in children of both sexes (Lamminmaki et al., 2012).

Disruption of the sex-typical early hormonal milieu alters subsequent play behavior. In animal models, castration at birth results in less masculine behavior in males (Goy and McEwen, 1980, Wallen, 2005), while prenatal androgen administration results in more masculine behavior in females (Sachser and Kaiser, 1996). This appears to be true in humans as well (Hines, 2003). Girls with congenital adrenal hyperplasia, in which enzymatic deficiencies lead to excessive androgen production starting early in fetal development, show more masculine patterns of toy and activity preferences than unaffected girls (Berenbaum and Hines, 1992, Meyer-Bahlburg et al., 2004), despite evidence they tend to be more strongly encouraged to pursue "feminine" play behaviors than unaffected females (Pasterski et al., 2005). Finally, perinatal exposure to environmental chemicals that interfere with gonadal hormone activity also affects sex-typical play behavior. Prenatal exposure to phthalates and perinatal exposure to polychlorinated biphenyls (PCBs) are both associated with less masculine play in boys during childhood (Swan, Liu, 2010, Vreugdenhil et al., 2002). In girls, on the other hand, perinatal PCB exposure is associated with more masculine play, whereas perinatal dioxin exposure is associated with more feminine play (Vreugdenhil, Slijper, 2002). The associations between PCB exposure and sexually dimorphic play behavior in boys and girls appear to be long-lasting, and are still evident at ages 6–8 (Winneke et al., 2013).

If stress acts as a non-chemical endocrine disruptor, altering androgen activity in the fetus, we predict that it will also affect sex-typed play behavior in childhood. To our knowledge, only one study to date has focused on the question of how prenatal stress affects play behavior in children, finding that after adjusting for a number of covariates, exposure to prenatal stress was weakly associated with more masculine play behavior in girls, but was not associated with play behavior in boys (Hines et al., 2002b). In these analyses, we attempt to replicate those findings in a second cohort, hypothesizing that prenatal exposure to life events stress is associated with more masculine play behavior in girls. Given the

inconsistency of findings between the animal and human studies, we have no *a priori* hypothesis regarding the association between stress and play behavior in males.

Methods

Study Population

The Study for Future Families (SFFI; 1999–2002) began as a multi-center pregnancy cohort study including women and their partners from four U.S. cities (Columbia, MO; Los Angeles, CA, Minneapolis, MN, and Iowa City, IA). In 2000, 474 infants born of those pregnancies were examined in a follow-up study, SFFII. In 2006, a second follow-up study (SFFIII) was conducted to examine play behavior in relation to prenatal exposures in the participating children. Eligibility criteria for SFFIII included: participation in SFFII, birth date from 2000–2003, and having a mailing address on record. Three hundred and thirty-four families were sent questionnaires, and of those, 128 families could no longer be reached at their listed addresses (questionnaires returned as undeliverable) and an additional 56 did not respond. Thus 150 questionnaires were ultimately completed and returned (Figure 1). It is this sub-population we focus on in the current analysis. All phases of SFF were approved at participating institutions prior to study implementation and all subjects signed informed consent for each study. Recruitment and study design and methods have been described elsewhere (Swan, Main, 2005).

Prenatal questionnaires

During the pregnancies, women completed an extensive questionnaire on general health, lifestyle, and reproductive history (mean gestational age: 25 weeks). Included in the questionnaire was a series of items on stressful life events occurring within the previous three months (or less) of pregnancy. Specifically, subjects were asked whether they had experienced: job loss or unemployment (self or partner), serious injury or illness (self or partner), death of a close family member (i.e. parent, child, sibling), divorce, separation, or serious relationship difficulties with one's partner; serious legal or financial problems (self or partner); or other major life events (open-ended, write-in option). These items were derived from standardly used questionnaires (Dohrenwend et al., 1978, Holmes and Rahe, 1967) and the life events selected were chosen based on criteria described elsewhere (Dohrenwend and Dohrenwend, 1978, Dohrenwend, Krasnoff, 1978). We then tallied the number of life events reported to create a maternal life events stress scale (ranging from 0–5 life events stressors reported). The questionnaires included additional items on race, age, employment, alcohol use, smoking, drug use, diet, and reproductive history.

Pre-School Activities Inventory (PSAI)

Parents were contacted by mail and asked to complete the PSAI, a widely validated, standardized tool designed to look at variation in children's play behavior (toys, activities, and personality) both within and between sexes (Golombok and Rust, 1993a, b). It is one of the few tools that can measure sexually dimorphic play behaviors in children as young as age 2. Parents or caretakers are asked to complete a series of 24 items on a 5-point, Likert scale (never, hardly ever, sometimes, often, very often). The items focus on types of toys, activities, and general child characteristics. Twelve items have been classified as "feminine"

whereas 12 have been classified as “masculine”. The PSAI is scored by subtracting the sum of the feminine items from the sum of the masculine items, and then applying a standard transformation factor (Golombok and Rust, 1993b)), such that a high composite score indicates more male-typical play and a lower composite score indicates more female-typical play. In this analysis, we also considered the masculine and feminine scales individually, and higher scores on each represent more male-typical and female-typical play, respectively.

Parental Attitude Scale and assessment of other familial covariates

In order to collect data on other factors that might influence a child’s engagement in gender-typical play, at the time of PSAI completion, parents were also asked to complete a brief questionnaire on the index child’s siblings (number, age, and sex), household composition (number, age, and sex of other adults), parental education, and parental attitudes about sex-atypical play. Mothers were asked, “What would you do if you had a boy who preferred toys that girls usually play with?” and “What would you do if you had a girl who preferred toys that boys usually play with?” They were asked to respond to each item on a 5-point Likert scale (1-strongly encourage, 2-encourage, 3-neutral, 4-discourage, 5-strongly discourage). They were then asked to report on how the child’s father would answer the same questions. The mother and father’s attitudes towards sex-atypical in boys were summed to create parental attitude- boys (PAB) scale, ranging from 2 (strongest encouragement of sex atypical play in sons) to 10 (strongest discouragement of sex atypical play in sons). An analogous scale (parental attitudes-girls, PAG) was created as the sum of mother’s and father’s scores of sex-atypical play in girls. These variables have been published upon before in this population and described in greater detail (Swan, Liu, 2010), but have not been validated.

Statistical Analysis

We first examined descriptive and summary statistics for relevant study variables, then looked at bivariate associations between them. Due to the distribution of reports of life events stressors during pregnancy, we dichotomized maternal stress as one or more life events (“stressed”) and zero life events (“low stress”) groups. All analyses, bivariate and multivariable, were stratified by sex of child given our *a priori* assumption that there would be sex differences in PSAI scores and that the effects of stress on PSAI scores might differ between the sexes. We used t-tests and calculated Cohen’s d to verify that there were sex differences in PSAI scores. We fit linear regression models to examine the association between prenatal exposure to life events stress and play behavior in childhood. We fit three models in each sex to look the association between prenatal life events stress and: (1) masculine PSAI score; (2) feminine PSAI scores; and (3) composite PSAI scores.

We considered a number of possible covariates for inclusion in these multivariable models: age of child, maternal age, paternal age, maternal education, paternal education, presence of opposite sex siblings in the home, presence of an adult male in the home, parental attitudes, race, ethnicity, alcohol use during pregnancy, and study center. Variables that were significant at an alpha-level of 0.15 or less or changed the estimate of the association between prenatal exposure to life events stress and PSAI scores by 10% or more were retained in final models. These included: child’s age, mother’s age, parental attitude score (PAG or PAB), having an opposite sex sibling (younger or older), and having an older, same

sex sibling. To address possible concerns about validity of maternal reporting of paternal attitudes towards sex atypical play, we conducted a sensitivity analysis, refitting all models to include maternal attitudes (rather than combined parental attitudes) as a covariate. Finally, for each of our models, we calculated Cohen's d to assess the effect size of prenatal stress on PSAI scores (Rosnow et al., 2000). As a rule of thumb, a Cohen's d of 0.2 is considered a small effect, 0.5 is considered medium, and 0.8 is considered a large effect (Cohen, 1988). The p-values reported are two-tailed, with an alpha level of p=0.05 unless otherwise noted. A p-value >0.05 but ≤0.10 was interpreted as indicating a trend. All analyses were performed using SAS Enterprise Version 4.3 (SAS Institute, Cary, N.C., USA).

Results

In total, 150 SFF mothers completed and returned questionnaires including the PSAI. Four of those women were excluded from the current analyses because of missing data. On average, women who returned questionnaires had higher education levels and were more likely to be Caucasian than women who could not be reached. Mothers who provided follow-up data for the current analyses also reported fewer life events stressors, on average, than women who did not participate in the current follow-up (not shown).

The study population was predominantly Caucasian with over half having at least some college education (Table 1). Nearly half of women (47%) reported no life events stressors during pregnancy and for the purposes of this analysis, were therefore classified as "low stress". The remaining 53% who reported one or more life events were classified as "stressed". The number of life events did not differ significantly between male and female gestations. In crude analyses, compared to the stressed group, women in the no life events stress group were more likely to have gone to college and have a partner who went to college, but the two groups did not differ in age, partner's age, race, or parental attitudes towards boys' (PAB) and girls' (PAG) play. On average, the index children were 4.9 years old at the time of PSAI completion.

PSAI scores were similar to those published in other populations (Table 2). As expected, on average, boys had higher masculine scores (mean=44.8; SD=6.5) than girls (mean=31.6, SD=6.0) (t=12.7; df=144 ; d=2.1). Girls had higher feminine scores (mean=47.9, SD=5.0) than boys (mean=30.3, SD=6.2) (t=18.9; df=144; d=3.1). As a result, as expected, composite scores were higher in males (mean=64.2; SD=8.1) than in females (mean=30.3; SD=7.7) (t=25.8; df=144; d=4.3)(Golombok and Rust, 1993b). In bivariate analyses, PSAI scores in girls and boys were correlated with a number of demographic and social factors (Table 3). In both sexes, parental attitudes towards sex atypical behavior showed the strongest correlation with composite PSAI scores (r= 0.30 for boys, r=−0.44 for girls). In addition, in boys, parental education and alcohol use during pregnancy were significantly correlated with PSAI scores, whereas in girls, maternal stress during pregnancy and having an older sister were most significantly correlated with PSAI scores. However, not all of these variables met criteria for inclusion in final multivariable models.

In multivariable models, girls born to mothers reporting one or more life events stressors during pregnancy had significantly higher masculine PSAI scores than girls whose mothers

reported no life events stressors during pregnancy (β=3.48, p=0.006) (Table 3). There was a similar trend towards higher composite scores (β=2.63, p=0.08) among daughters of stressed mothers as well. There were no stress-related differences in feminine scale scores in girls. In boys, masculine and composite scores were not associated with maternal prenatal stress. However sons of women reporting one or more stressors during pregnancy showed a trend towards higher scores on the feminine PSAI scale (β=2.23, p=0.10). In our calculations of effect size, in girls, the d for maternal stress was very small for the PSAI feminine sub-scale (0.08), but of medium size for the masculine and composite scores (0.62 and 0.50, respectively). Among boys, the magnitude of the effect size for maternal stress was negligible for the PSAI masculine subscale and of small size for the feminine and composite scales (0.23 and 0.20, respectively) (Table 3). In sensitivity analyses using maternal attitudes about sex-atypical play (rather than combined parental attitudes) as a covariate, the results were unchanged (not shown).

Discussion

Our results show that the association between exposure to prenatal life events stress and play behavior in childhood (as assessed by the PSAI) differs by child's sex. Girls exposed to prenatal life events stress had significantly higher masculine PSAI scores than unexposed girls and showed a trend towards a higher (more masculine) composite score. Conversely, boys exposed to prenatal life events stress showed a non-significant trend towards higher feminine PSAI scores than unexposed boys, with no differences on the masculine or composite scales. Furthermore, the effect sizes were larger in girls than in boys. These results are largely consistent with animal models (Dahlof et al., 1977, Sachser and Kaiser, 1996, Ward, 1972, Ward and Stehm, 1991) as well as our related work on prenatal stress and other sexually dimorphic outcomes (Barrett, Parlett, 2013). This body of results suggests that prenatal stress is not only as a possible modifier of the effect of environmental chemicals on fetal development, but also may itself be a non-chemical endocrine disruptor in humans.

The current results are consistent with the only similar published study (in a much larger study of British children), in which prenatal stress was associated with higher (more masculine) composite PSAI scores in girls. That study did not report the masculine and feminine scores individually so we cannot directly compare the estimates for the sub-scales (Hines, Johnston, 2002b), however, the consistency across two studies with different populations and methods speaks to the robustness of the results in females. In both studies, there was no association between prenatal stress and overall, composite PSAI score in boys. It would be of interest to examine whether scores on the feminine sub-scale varied in relation to prenatal stress in boys in that study, as they did in ours, given the greater power afforded by the larger sample size. It should be noted that only the full PSAI has been validated, however our results regarding the masculine and feminine sub-scales suggest a need for further investigation of this question using inventories intended to assess masculine and feminine behaviors separately.

These results are interesting in light of our findings on the sexually dimorphic effects of prenatal stress on AGD, a marker of prenatal androgen exposure that is widely used as a

measure of reproductive toxicity. In that analysis, we found a similar pattern of results, whereby exposure to prenatal life events stress was associated with longer AGD in girls and a trend towards shorter AGD in boys (Barrett, Parlett, 2013). It is worth noting that unlike sexually dimorphic play behavior, which is almost certainly affected by factors in the postnatal environment (Rust et al., 2000, Weissner et al., 1994), AGD in infancy is likely to be primarily a product of the prenatal environment and genetics (Sathyanarayana et al., 2012), with relatively little postnatal, environmental contribution (Papadopoulou et al., 2013). Indeed, the fact that these two sets of findings are similar suggests that there may be a single mechanism by which prenatal stress can exert masculinizing effects on multiple body systems in females, and under-virilizing effects in males.

To this end, we suggest that prenatal stress may induce changes in fetal androgenic exposure, which may subtly masculinize developing females. At least one study has found that stressing pregnant rats results in highly elevated circulating testosterone levels in the mothers, presumably due to increased adrenal stimulation (Beckhardt and Ward, 1983). Whether these elevated maternal testosterone concentrations would be enough to overwhelm the placental aromatase that normally converts androgens to estrogens (thereby blocking the virilization of female fetuses) is unknown, but seems unlikely (McClamrock and Adashi, 1992). A second possible, non-mutually exclusive mechanism is that heightened stress allows maternal cortisol to cross the placenta more readily, thereby affecting fetal adrenal, ovarian, and/or testicular function. Although maternal cortisol is much higher than fetal cortisol in normal pregnancies, the two are highly correlated ($r=0.62$, $p<0.001$) (Gitau et al., 1998). Increasingly, evidence suggests that under high stress conditions, not only may maternal cortisol levels rise, but placental enzymes which normally inactivate cortisol, preventing it from reaching the fetus (notably 11β-hydroxysteroid dehydrogenase, type 2, or 11β-HSD2), may be dysregulated (Mairesse et al., 2007, O'Donnell et al., 2012). Some evidence suggests that production of 11β-HSD2 is more responsive to environmental cues in the female placenta than the male placenta (Clifton, 2010). We have previously hypothesized that the resulting, increased fetal exposure to cortisol may then affect fetal or placental androgen activity (Barrett, Parlett, 2013), and that the association between the two hormones may be stronger in female than male fetuses (Bergman et al., 2010, Sarkar et al., 2008).

Indeed, stress appears to affect androgenic activity not only in the mother and the placenta, but in the fetus as well. In animal models, prenatally stressed female fetuses have increased testosterone levels compared to controls (Ward and Weisz, 1980, 1984). In prenatally stressed male fetuses, the relationship is less clear-cut, although there appears to be blunting of the male-typical prenatal testosterone surge during the “male programming window” in early pregnancy (vom Saal, Quadagno, 1990, Ward and Weisz, 1980, 1984) and testicular production of testosterone is lower in prenatally stressed male rat pups (Ward and Weisz, 1980). It is possible that prenatal stress stimulates androgen production by the fetal adrenal glands (site of approximately 50% of female androgen production)(Longcope, 1986), while inhibiting fetal testicular androgen production (Dahlof, Hard, 1978), thereby producing opposite effects in males and females. Because early PCB exposure also appears to elicit the same sexually dimorphic effects on PSAI-measured play behavior as stress (higher exposure associated with more masculine scores in girls and more feminine scores in boys),

investigating possible common mechanisms of action on androgenic and estrogenic pathways may be informative (Golden et al., 1998).

Unlike synthetic environmental chemicals, stress is part of our evolutionary history and thus it is possible that stress-related changes in neurodevelopment confer an adaptive advantage to affected offspring (Glover and Hill, 2012). Theoretically, if prenatal environment is the best indicator of the quality of the postnatal environment, then fetal exposure to prenatal stress may yield valuable information that could produce a competitive advantage under harsh or changing conditions. In rodents, although prenatally masculinized females are less attractive to males and have lower reproductive success than controls under normal conditions, in a high-density, stressful environment the same females actually have higher reproductive success. The difference in reproductive success under those conditions is due to increased risk of death among offspring born to the less aggressive control females (vom Saal, 1981). Female macaques born to stressed mothers exhibit better developed visual orientation patterns at one day of age than females born to non-stressed mothers, whereas the opposite is true of male offspring (Novak and Sackett, 1996). Finally, a small psychological literature suggests that this question merits further investigation in humans. Several studies have linked childhood stressors to earlier age at menarche, suggesting that early exposure to stress could program an accelerated reproductive trajectory in females, though we know of no studies that have looked at early exposure to stress in relation to reproductive function or success past menarche (Belsky et al., 1991, Buzney and DeCaro, 2012, Ellis and Garber, 2000, Wierson et al., 1993). Similarly, we know of no studies that have examined *prenatal* stress in relation to timing of reproductive trajectories, however the question merits further study.

There are some limitations to the current study. First, our sample size was clearly small and may have impaired our ability to detect associations and draw valid conclusions about the relationship between prenatal stress and postnatal play behavior, particularly in boys. That we had similar sample sizes of boys and girls, but only found statistically significant relationships in the latter suggests that there is a stronger relationship between maternal stress and subsequent sexually dimorphic play behavior in girls than in boys. Whether there is truly any association in males requires additional research in a larger cohort, and the results of at least one previous study suggest that there may not be (Hines, Johnston, 2002b). In addition, it is worth noting that any childhood behavior in humans, including play behavior, is likely to be influenced by numerous other factors in the postnatal environment. Even at very young ages, parental “gender-typing” may influence children’s sexually dimorphic play and gender-related attitudes (Tenenbaum and Leaper, 2002, Zosuls et al., 2009). Play behavior also varies in relation to the sex of a child’s playmates, suggesting that the sex of siblings is important (Fabes et al., 2003). Indeed, recognizing these postnatal influences, we collected data on many possible predictors of postnatal play behavior. That we were able to collect data on a number of important predictors of play behavior is a notable strength of our study, and several of these predictors, including presence and sex of siblings in the home, parental attitudes towards gender-specific play, child’s age, and mother’s age at conception, were included in our final models. The previous, related British analysis also adjusted for maternal use of alcohol and tobacco, as well as maternal education

(Hines, Johnston, 2002b), although these factors were not important predictors in our multivariable analyses. That study ultimately concluded that although prenatal stress predicted increased masculine play behavior in girls, other factors (most notably sex of siblings and parental adherence to traditional sex roles) were more important predictors, and other studies have echoed the importance of older siblings in gender development (Rust, Golombok, 2000). Our study found, similarly, that girls' PSAI scores were most strongly correlated with parental attitudes and that prenatal stress and having an older sister showed slightly weaker correlations with PSAI scores (Table 3). Thus in both studies, the same patterns emerged and the main findings persist after adjusting for known covariates and confounders, but the possibility remains that other, unmeasured factors could underlie the relationships reported here.

It is also worth noting that the population studied was not highly stressed and by design, all women participating had partners who started the study with them. We also had higher rates of loss to follow-up among stressed women. Therefore, we cannot necessarily extrapolate our results to a more highly stressed population with less social support. However, these results suggest that additional research in demographically varied cohorts is warranted. In addition, we recognize that calling women who reported one or more life events "stressed" and those who reported no life events "low stress", may be inaccurate and doesn't capture other types of stressors, such as daily hassles. We did not incorporate individual variation in perception of and response to life events stressors. However, in the 2002 British study, researchers assessed number of life events stressors as well as the perceived impact of those stressors, finding that the relationship with PSAI scores was nearly identical (Hines, Johnston, 2002b). Finally, we and others propose that the noted sexually dimorphism in play is in part, the product of hormones organizing neural anatomy and physiology at critical points in prenatal development, yet it is unclear exactly when those critical periods are. As our understanding of mechanisms and temporal relationships improves, we can begin to more accurately assess stress at those precise, sensitive time points, rather than measuring stress so broadly across all of pregnancy.

Clearly, questionnaire-based parental reports of play behavior have inherent limitations. Ideally, one might observe play behaviors in an observational, laboratory setting, rather than relying on parental reports of play behavior which may be biased by parental preferences or expectations (Golombok and Rust, 1993a). However parental reports have the advantage that they capture typical behaviors across time and context (rather than just during a brief observation) (Golombok and Rust, 1993a). Furthermore, the PSAI offers obvious logistic advantages over in person observations and its validation allows for widespread use and comparison across many populations and studies (Hines, Golombok, 2002a, Hines, Johnston, 2002b, Lamminmaki, Hines, 2012, Pasterski, Geffner, 2005, Swan, Liu, 2010, Vreugdenhil, Slijper, 2002), although some have questioned its validity (Kaufman, 2003). Nevertheless, we propose that attempting to replicate the current results using an experimental play paradigm (rather than using the PSAI) will be an important test of epidemiological consistency and should be a priority for future work in this area. Finally, more research is needed to determine whether exposure to prenatal stress is associated with additional sexually dimorphic traits later in childhood as well as in adulthood, particularly those that are known to be sensitive to prenatal endocrine disruption.

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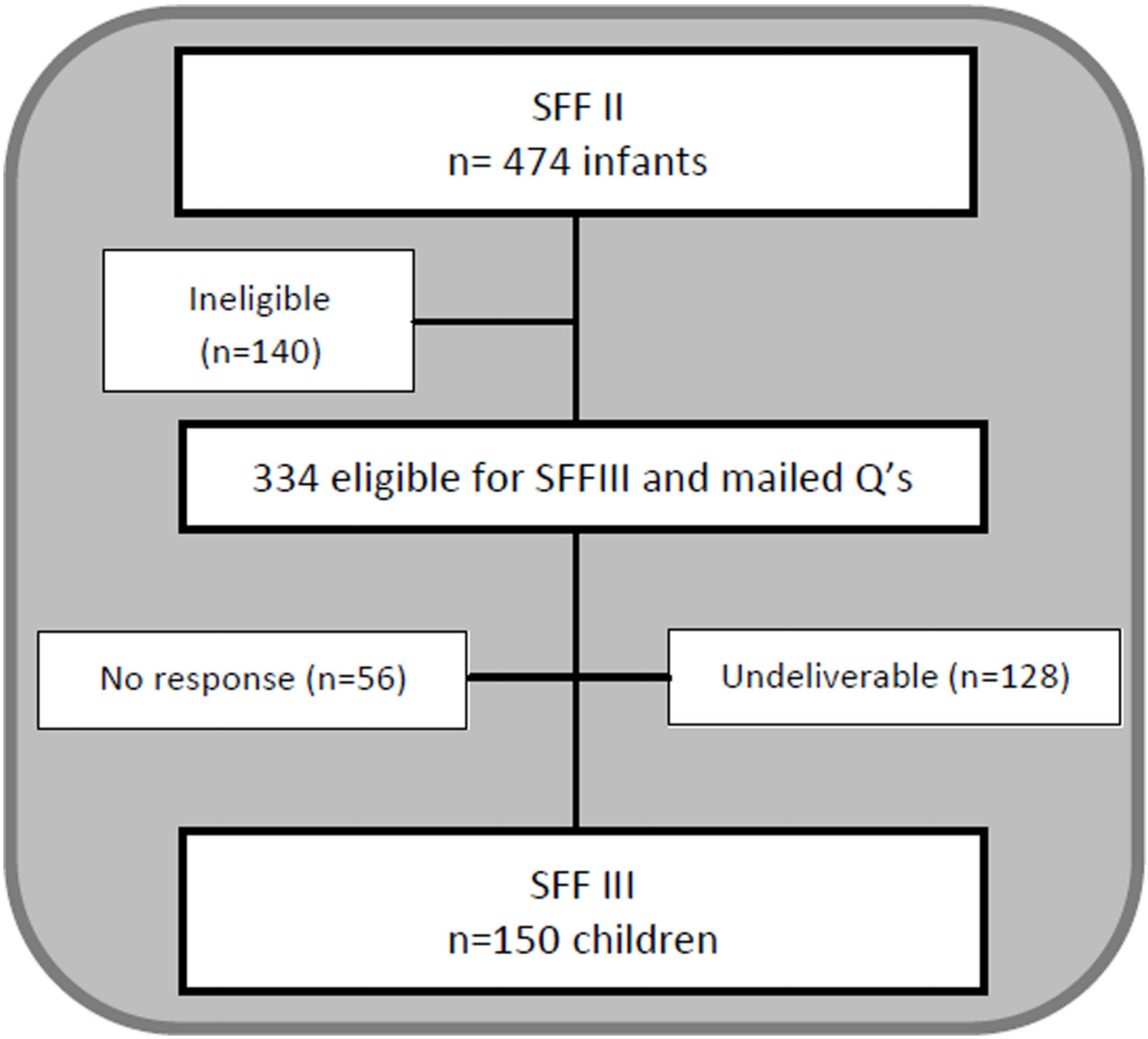

Figure 1.
Overview of study recruitment for SFFIII.

Table 1

Demographic characteristics and anthropometrics of the SFF subjects by parental life events stress category[a] (n=146).

	Number of stressful life events during pregnancy (as reported by mother)	
	None (n=69)	One or more (n=77)
Characteristic	**N(%)[a]**	**N(%)[a]**
Child's sex		
Male	33 (48)	41 (53)
Female	36 (52)	36 (47)
Race		
White	63 (91)	65 (84)
Other	6 (9)	12 (16)
Maternal education		
College or more	55 (80)	51 (66)
Paternal education		
College or more	55 (80)	36 (47)
Maternal alcohol use during pregnancy (any)	0 (0)	4 (5)
Has opposite sex sibling	39 (56)	40 (52)
Older opposite sex sibling	17 (25)	19 (25)
Has same sex sibling	23 (33)	25 (32)
Older same sex sibling	10 (24)	9 (20)
Study Center		
California	3 (4)	11 (14)
Iowa	17 (25)	10 (13)
Minnesota	28 (41)	32 (42)
Missouri	21 (30)	24 (31)
	Mean (Min-Max)	**Mean (Min-Max)**
Child's Age (months) at PSAI	59.0 (42.7–72.0)	59.2 (42.7–77.3)
Maternal Age at Birth	30.4 (20.7–39.3)	30.5 (18.3–41.3)
Paternal Age at Birth	32.0 (21.5–41.8)	32.6 (18.2–47.7)
Parental attitude score- Boys (PAB)	6.2 (4–10)	6.2 (2–10)
Parental attitude score- Girls (PAG)	5.4 (2–8)	5.3 (2–9)
Number of children in household	2.4 (1–5)	2.3 (1–6)

[b] Percentages may not add up to 100 due to rounding.

Table 2

PSAI scores in SFF subjects compared to published data from Golombok et al (2008) and Hines et al. (2003).

	SFF	Golombok et al. (2008)	Hines et al. (2003)
Boys			
N	74	2726	4863
Mean age (months)	59	57	42
Mean PSAI scores (SD)			
Composite	64.2 (8.1)	64.2 (8.8)	62.4 (0.125)[a]
Masculine sub-scale	44.8 (6.5)	*	*
Feminine sub-scale	30.3 (6.2)	*	*
Girls			
N	72	2775	4531
Mean age (months)	59	57	42
Mean PSAI scores (SD)			
Composite	30.3 (7.7)	35.1 (9.4)	36.9 (0.14)[a]
Masculine sub-scale	31.6 (6.0)	*	*
Feminine sub-scale	47.9 (5.0)	*	*

[a] Standard error of the mean, not SD, was reported in this study.

* Data not reported.

Table 3

Correlations between PSAI scores and predictors for boys and girls. Associations significant at p≤0.05 are bolded.

	PSAI Scores; Correlation coefficient (r)					
	Girls (n=72)			Boys (n=74)		
	Composite	**Masculine**	**Feminine**	**Composite**	**Masculine**	**Feminine**
Age of child	0.02	−0.10	−0.16	0.24	0.08	−0.20
Race	0.03	0.09	0.06	−0.15	−0.02	0.16
Maternal age	0.03	0.20	0.21	0.14	−0.01	−0.18
Paternal age	0.07	0.20	0.16	0.17	−0.05	**−0.26**
Maternal education	0.10	0.16	0.06	0.27	0.05	**−0.28**
Paternal education	0.03	−0.04	−0.09	−0.10	−0.10	0.01
Alcohol use during pregnancy	0.11	0.19	0.07	**0.24**	**0.22**	−0.06
Maternal stress during pregnancy	**0.24**	**0.30**	0.03	−0.10	0.002	0.12
Any opposite sex sibling	0.20	0.20	−0.03	−0.04	−0.16	**0.21**
Older, same sex sibling	**−0.28**	−0.12	**0.26**	−0.01	0.05	0.03
Parental attitudes on atypical play behavior in boys (PAB)	-	-	-	**0.30**	0.10	**−0.25**
Parental attitudes on atypical play behavior in girls (PAG)	**−0.44**	**−0.36**	0.14	-	-	-
Adult male in the home	0.05	0.02	−0.04	0.11	0.13	0.01

Table 4

Associations between prenatal life events stress and PSAI scores in multivariable models.

	Female (n=72)[a]					Male (n=74)[b]				
PSAI score	**Difference[c]**	**Lower 95%**	**Upper 95%**	**p-value**	**Cohen's d**	**Difference[c]**	**Lower 95%**	**Upper 95%**	**p-value**	**Cohen's d**
Composite	2.63	−0.41	6.02	0.08	0.50	−1.44	−5.15	2.26	0.44	0.20
Masculine sub-scale	3.48	1.04	5.93	0.006	0.62	0.92	−2.86	4.15	0.71	0
Feminine sub-scale	1.09	−1.12	3.29	0.32	0.08	2.23	−0.51	4.97	0.10	0.23

[a] Models also include: child's age, mother's age at conception, parental attitudes towards girls' play, having a brother, and having an older sister.

[b] Models also include: child's age, mother's age at conception, parental attitudes towards boys' play, and having a sister, and having an older brother.

[c] Difference between children born to women reporting 1+ life events stressors during pregnancy versus children born to women reporting zero life events stressors during pregnancy.