[Frontiers in Bioscience E2, 43-46, January 1, 2010]

Maternal stress-induced reduction in birth weight as a marker for adult affective state

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1. ABSTRACT

It is known that adverse events experienced by a pregnant woman may be reflected upon the developing fetus and adversely affect its mental wellbeing in later life. In a recent study by our group, prenatal stress was associated with a clear increase in anxiety- and depression-related behavior in male, but not female Sprague-Dawley offspring. Since birth weight data were recorded we were able to determine whether birth weight, as an important outcome measure of fetal distress, may be used as a predictive indicator for adult performance. For this purpose, a correlation analysis was performed, aimed at studying the possible link between stress-induced fetal growth restriction and adult affective state. Male birth weight correlated positively to depression-related behavior in the forced swim test. Furthermore, it weight was correlated negatively to basal, and positively to stress-induced, plasma corticosterone levels in adulthood. Female birth weight did not correlate to any of the studied outcome measures. These data suggest that male birth weight may represent a valuable indicative marker for variations in adult affective state with a developmental origin.

2. INTRODUCTION

Nowadays, it has become increasingly clear that prenatal stress (PS) i.e., stress experienced before birth, influences the development of an individual *in utero* and adversely affects its mental and physical wellbeing in later life. In humans, for example, PS has been associated with the development of various cognitive and affective disorders such as depression and anxiety (1-3).

In a recent study we examined the effects of PS on anxiety- and depression-related behavior in adult male and female Sprague-Dawley rats (4). In that investigation, PS was associated with a clear increase in anxiety-related behavior in male, but not female offspring, as evaluated in the elevated zero maze and the home cage emergence test. Likewise, depression-related behavior in the forced swim test was increased in PS male rats only. PS male offspring further showed increased basal plasma corticosterone levels, whereas both PS males and females failed to show an adequate response to stress with lower stress-induced corticosterone levels as compared to controls.

Since all pups were weighted at birth we were able to determine whether birth weight, as an important outcome measure of fetal distress, may be used as a predictive marker for adult performance in the abovementioned tasks. For this purpose, a correlation analysis was performed, aimed at studying the possible link between stress-induced fetal growth restriction and adult performance.

3. MATERIALS AND METHODS

3.1. Animals and Procedures

Part of the data were derived from a larger, ongoing study (4). This study was approved by the Animal Ethics Board of the University of Maastricht, The Netherlands. Acclimatized Sprague-Dawley rats (Charles River, The Netherlands) were used. The animals were housed individually within a temperature-controlled environment (21±1 degrees C) with a 12 h light/12hr dark cycle (lights on from 0700 - 1900 h) and had access to standard rat chow and water *ad libitum*. Pregnancy was determined by observation of vaginal plugs (embryonic day 0 – E0). Restraint stress was performed daily during the last week of pregnancy (E14-E21). Pregnant female rats (n=8) were individually restrained 3 times a day for 45 minutes per session (at approximately 0900 - 0945 h, 1300 - 1345 h, and 1700 - 1745 h) in transparent plastic cylinders while at the same time being exposed to bright light (5). Control (C) pregnant females (n=8) were left undisturbed in their home cages. Within an hour after the last pup of a litter had been born, gender (based on anogenital distance) and individual body weights were determined, and pups were individually labeled by means of toe cut. Only litters of 8 or more pups were included in this study. Litters were culled to 8 pups (if necessary). At postnatal day 21 (P21), pups were weaned and housed together (2 male or 2 female rats/cage; n=14 rats per experimental condition per gender) for further examination. Rats were kept at a reversed day-night cycle from

Table 1. Dam weight (g) over gestation

Group	E0	E21	P21
C	270.7 ± 3.7	431.4 ± 6.1	349.9 ± 3.0
PS	268.3 ± 3.1	393.0 ± 7.5[2]	317.4 ± 11.3[1]

Values represent means ± S.E.M. Abbreviations: E: Embryonic day, P: postnatal day, C: control, PS: prenatal stress group; [1]P<0.05, [2]P<0.01 (Student's t-test).

Table 2. Birth weight (g)

Gender	Group	P0
Males	**C**	**7.0 ± 0.2**
	PS	**6.1 ± 0.2[3]**
Females	**C**	**6.6 ± 0.1**
	PS	**5.9 ± 0.2[3]**

Values represent means ± S.E.M. Abbreviations: C: control, PS: prenatal stress; [3]P<0.001 (Student's t-test).

this point onwards (lights on from 1800 - 0600 h). Anxiety- and depression-related behavior of the rats was analyzed from P120 onwards using the elevated zero maze, the home cage emergence test, the modified forced swim test as described in detail previously (4). In addition, hypothalamo-pituitary-adrenal (HPA) axis reactivity was examined by determining stress-induced plasma corticosterone secretion using a radioimmunoassay.

3.2. Statistical Analysis

Body weights of the pregnant dams were analyzed using a repeated measurements ANOVA (experimental condition x time) and also independently at the various time points using a Student's t-test. Birth weight of the offspring was analyzed using a Student's t-test. Correlation analysis was performed using Pearson's correlation coefficient (r_p). Statistical significance was assumed to exist at P<0.05. All statistics were carried out using SPSS software version 12.0.1 (SPSS Inc, USA). A maximum of 2 male and female pups per litter were examined to prevent litter effects (6).

4. RESULTS

Dam weight over time is depicted in (Table 1). A within-subjects effect was observed for time (P<0.001). Further, over time, an effect of maternal stress was observed (P=0.027). At E21 stressed dams had lower body weights as compared to controls (stress effect: -8.9%, P=0.002). At P21 dams from the PS group still showed reduced body weight as compared to controls (stress effect: -9.2%, P=0.031).

Birth weight data are shown in (Table 2). PS male rats weighted less at birth as compared to controls (PS effect: -12.9%, P<0.001). Similarly, PS females were lighter than control females (PS effect: -11.6%, P=0.001).

Correlations between the different parameters are depicted in (Table 3). In males, birth weight correlated positively to strong mobility (SM) in the forced swim test (r_p=0.475' P=0.011), i.e., low-birth-weight male offspring showed more depression-related behavior in adulthood. Further, male birth weight correlated negatively to basal plasma corticosterone levels (r_p= -0.463; P=0.015). Moreover, it correlated positively to stress-induced corticosterone levels (r_p=0.552; P=0.003, respectively), indicative of an affected stress response. In addition, within males, basal and stress-induced plasma corticosterone values were correlated to various types of behavior in the anxiety- and depression-related tasks [see (Table 3) for more details].

5. DISCUSSION

In a recent study by our group, prenatal maternal stress was associated with an increase in anxiety- and depression-related behavior particularly in male Sprague-Dawley rat offspring (4). PS male offspring further showed increased basal plasma corticosterone levels, whereas both PS males and females failed to show an adequate response to stress with lower stress-induced corticosterone levels as compared to controls. We now show that male birth weight in these same animals, which was reduced in reaction to PS, was a predictive marker both for depression-related behavior in the forced swim test, as well as for plasma corticosterone levels in adulthood. In addition, within males, basal and stress-induced plasma corticosterone levels were correlated to performance in both anxiety- and depression-related behavioral tasks.

The 'Developmental origins of health and disease' (DOHAD) concept (7) states that the risk of disease in adulthood partly depends upon variations in the prenatal environment, which is often reflected in body weight at birth (8). Fetal undernutrition, for example, resulting in impaired fetal growth, predisposes individuals to the development of cardiovascular disease, insulin resistance and non-insulin-dependent, type 2 diabetes (9, 10). Further, reduced birth weight has been linked to an increased susceptibility to stress (11, 12) and affective disorders in later life (13-18). Interestingly, in the present study, a significant positive correlation between male fetal growth and strong mobility in the forced swim test was observed. In addition, male birth weight was correlated negatively to basal, and positively to stress-induced plasma corticosterone levels in adulthood.

The relationship between fetal growth and disease risk in later life reflects the sensitivity of fetal growth to adverse intrauterine influences and does not imply a contributory role of being born small (19). In this respect, birth weight is a rough integrated measure of many fetal processes and various intrauterine adverse events may have independent effects on fetal growth while in parallel also having long-term pathological consequences. The observed reduction in birth weight in PS pups in the present study may be explained by a reduction in food and water intake and an impaired conversion of dietary calories into maternal weight gain as seen in reaction to stress (20, 21). In support of this idea, maternal weight gain over gestation was reduced by restraint stress. Further, the transplacental transport of maternal corticotrophin-releasing factor (CRF) and corticosterone, and excess sympatho-adrenal activation resulting in a reduction in uteroplacental blood flow may impair fetal growth (3, 20, 22).

Although the overall prevalence of mood disorders is higher in females as compared to males (23), a different pattern seems to be observed in PS-related psychopathology (24). Whereas only male PS Sprague-Dawley rats showed a clear increase in anxiety- and depression-related behavior, PS females seemed to remain largely unaffected, which is in line with other studies on PS

Table 3. Correlations between the different parameters studied in males (A) and females (B)

A: males											
	PS effect	**BW**	**EZM-OA**	**EZM-DM**	**HCE-EL**	**FS- IM**	**FS-M**	**FS- SM**	**CORT-B**	**CORT-S**	**CORT-R**
BW	↓		-	-	-	-	-	.48[1]	-.46[1]	.55[2]	-
EZM-OA	↓	-		.45[1]	-.48[1]	-	-	-	-	.48[1]	-
EZM-DM	↓	-	.45[1]		-	-	-	-	-	.59[2]	-
HCE-EL	↑	-	-.48[1]	-		-	-	-	-	-.46[1]	-
FS-IM	=	-	-	-	-		-	-.72[3]	-	-	-
FS-M	=	-	-	-	-	-	-	-	-	-	-
FS-SM	↓	.48[1]	-	-	-	-.72[3]	-		-.43[1]	.39[1]	-
CORT-B	↑	-.46[1]	-	-	-	-	-	-.43[1]		-.39[1]	-
CORT-S	↓	.55[2]	.48[1]	.59[2]	-.46[1]	-	-	.39[1]	-.39[1]		-
CORT-R	=	-	-	-	-	-	-	-	-	-	
B: females											
	PS effect	**BW**	**EZM-OA**	**EZM-DM**	**HCE-EL**	**FS- IM**	**FS-M**	**FS- SM**	**CORT-B**	**CORT-S**	**CORT-R**
BW	↓		-	-	-	-	-	-	-	-	-
EZM-OA	=	-		-	-	-	-	-	-	-	-
EZM-DM	=	-	-		-	-	-	-	-	-	-
HCE-EL	=	-	-	-		-	-	-	-	-	-
FS-IM	=	-	-	-	-		-	-.59[2]	-	-	-
FS-M	=	-	-	-	-	-	-	-	-	-	-
FS-SM	=	-	-	-	-	-.59[2]	-		-	-	-
CORT-B	=	-	-	-	-	-	-	-		-	-
CORT-S	↓	-	-	-	-	-	-	-	-		-
CORT-R	=	-	-	-	-	-	-	-	-	-	

Depicted are Pearson's correlation coefficients (r_p) representing the associations between the various parameters. The second column on the left summarizes the main effects of prenatal stress (PS). Abbreviations: BW: birth weight, EZM-OA: elevated zero maze; time spent in open arms, EZM-DM: elevated zero maze; distance moved,HCE-EL: home cage emergence; escape latency, FS-IM: forced swim test; immobility, FS-M: forced swim test; mobility, FS-SM: forced swim test; strong mobility, CORT-B: basal plasma corticosterone levels, CORT-S: stress-induced plasma corticosterone levels, CORT-R: plasma corticosterone levels after 40 minutes of recovery, [1]$P<0.05$; [2]$P<0.01$; [3]$P<0.001$. For more details on the listed parameters, see (4).

using repetitive restraint stress in pregnant Sprague-Dawley rats (24, 25). We now show that these gender-dependent effects can be traced back to the degree of fetal growth retardation in reaction to maternal stress. The gender-dependent effects of PS are probably related to the gender-specific timing of relevant developmental processes over gestation [e.g. (26)]. All in all, we postulate that patients suffering from affective disorders with a fetal/developmental origin may represent a different (sub)population –comprising primarily male subjects–, distinct from those –mostly female– subjects that develop an affective disorder in reaction to adult stressful life-events only. The finding that male, but not female, fetal growth is associated with adult performance supports this notion. The exact role of gender in relation to PS remains to be elucidated though and may largely depend on the genetic background of the subjects involved (25).

In conclusion, the present study shows that changes in adult depression-related behavior and HPA axis (re)activity induced by prenatal maternal stress can be predicted by the maternal stress-induced effects on fetal growth. The present findings suggest that birth weight may be useful as a predictive marker in PS-related investigations.

6. ACKNOWLEDGEMENT

Daniël L.A. van den Hove, Gunter Kenis and Jos Prickaerts were supported by European Union Framework Integrated Project NEWMOOD Grant LSHM-CT-2004-503474.

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Abbreviations: C: control; CRF: corticotrophin-releasing factor; DOHAD: developmental origins of health and disease; E: embryonic day; HPA: hypothalamo-pituitary-adrenal axis; P: postnatal day; PS: prenatal stress; SM: strong mobility (as measured in the forced swim test)

Key Words: Prenatal Stress, Depression, Anxiety, Birth Weight, Fetal Growth Restriction, Barker Hypothesis, Developmental Origins Of Health And Disease, DOHAD

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