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In utero Immobilization Stress and its Effects on the Development, Behavior and Sexual Maturity of the Rat

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The effect of prenatal stress from psychophysical immobilization, the applied during gestation, has been studied as well as its consequences on the development, behavior and sexual maturity in the offspring.

Modifications in development were slight, even when intensified at four months of age. Whereas male sexual behavior showed only slight variation, female sexual maturity was markedly changed in experimental animals as compared to controls. From the present results, the psychopathological action from immobilization stress in utero on offspring is questionable.

Studies carried out in various laboratories indicate that certain forms of stress in pregnant animals produce alterations in the development of the offspring. These include: decrease in size and viability of the litter (12, 16), delay (6, 14) or modification (6) in body weight, changes in cerebral proteins, nucleic acids and enzymatic activity (14), increase in cerebral catecholamines (9), abnormal adrenocortical activity (1) and behavioral changes (1, 10, 12, 18, 23).

In regard to sexual behavior, WARD (19) showed that the offspring of rats exposed to periodic stress displayed and abnormal model of adult sexual behavior. The action of prenatal stress on the behavior of the future male adult appears to be intimately linked with the adrenal and testicular release of androgens; such release by the fetus is dependent on the maternal hormones which cross the placenta, as well as on the fetus own hormones (20). It is likely that one or more of the hormones produced by the stress-for example, adrenalin and adrenocorticoids-cross the placental barrier in increased quantities due to the external events and alter the normal or effective ratio of fetal androgens. The final result is a detectable change in differentiation in those areas of the brain responsible

for adult sexual behavior (21). Indeed, male offspring whose mothers have suffered stress during pregnancy show lower levels of testosterone on the 18th and 19th days after conception than do controls (22).

Adult female rats who have suffered prenatal stress show an increase in the concentrations or the neurotransmiter dopamine in the hypothalamus and the arcuate nucleus. This increase has been associated with abnormalities in the release of gonadotrophic hormones from the hypophysis, determining in turn alterations in the estrous cycle and in reproductive capacity. Similar findings were noted by ROWEL (15) in baboons and by HOWARTH and HAWK (8) in sheep. It as also been postulated that the alterations noted in reproductive processes are due not only to the action of gonadotrophin secretion, but also to luteal or placental secretions, or to uterine blood flow. Thus, the administration of ACTH to pregnant rats causes and early senescence of the placenta (2); the same effect is noted upon administration of glucocorticoids (3). These changes in the placenta may secondarily affect the passage of oxygen and nutrients to the fetus in general, and to the brain in particular.

In this work we study the effect of immobilization stress during pregnancy offspring in the rat. We analyze the effects on the weight and number of neonates, and on the subsequent evolution of weight. Furter, we demonstrate the alterations in male sexual conduct, as well as possible modifications in female fertility, all with a view to defining the implications of this weak stress agent.

Materials and Methods

Wistar rats from our Department were subjected to standard conditions of light (12D, 12L), temperature (23 ± 3° C) and absolute humidity, with free access to food and drink.

Males and females were selected on the basis of their sexual maturity. Females were chosen from those who had perfect estrous cycles (4-5 day duration) and had not been in previous contact with males. Males were chosen according to their age, four months, and their potential for realizing sexual behavior and achieving ejaculation.

Pairing was achieved by placing three females and one male in the same cage at 5 P.M. and removing the male at 9 A.M. the following day. Copulation was verified on the basis of the presence of spermatozoids in the vagina. Pregnant females were separated at randon into two groups, one control (seven animals) an one experimental (six animals).

Stress induction was caused by introducing the animal into a plastic tube with several holes to facilitate breating. The tube was well adapted to the animal's body size and tall position. They were kept in this position for three hours, always beginning at 9 A.M. This stress agent was imposed on alternate days, with exposure time increased by 15 minutes with each session to avoid possible adaptation, up until the day of delivery.

All females carried to term, with no significant disturbances or abortions as a consequence of the stress, and all survived the experimental period. The litters of all females who carried to term were weighed and counted at birth and after one month. The age of vaginal opening was recorded in all animals, experimental and control. Their sexual cycle was also observed by means of vaginal citology taken for at least 20 consecutive days. In addition, this generation of females was mated with intact males selected at randon, to observe their percentage of pregnancy and the effects of the immobilization stress on the weight and number of their offspring. The sexual behavior of all, experimental and control, three month old males with

females injected 48 hours previously with estradiol benzoate, to insure sexual receptivity was observed.

The sexual behavior test were carried out by the method of SOULAIRAC (17). Behavior was observed in a 62.5×60.5×30 cm box containing five males and four females. One hour was the standard test lenght and the following parameters were recorded: —Number per minute of intromissions: Intromission is characterized by both the insertion of the penis into the vagina and a certain number of pelvic movements. The former are always followed by the licking by the male of its own penis and the sudden withdrawal of the female; whereas the latter are so-named because there is only pelvic movements no insertion of the penis into the genital canal of the female.

—Number of ejaculations: Ejaculation occurs after a certain number of intromissions. There is the observed a phenomenon of motor activity characterized by widening of the forelegs of the male and a shriek of the females.

—Refractory period measurement: This consists of a postejaculatory rest phase of variable duration during which the animal shows no sign of sexual activity and also displays complete motor inhibition.

Neuromotor activity: This parameter is represented by the relationship between the number of intromissions preceding ejaculation and the latency of ejaculation; that is, the time in minutes from first intromission untill the ejaculation occurs (f + v/Le). It permits the objective evaluation of the variation in the neuromotor activity.

At the moment of sacrifice, 4th months of age, was determined the weight (expressed in % of body weight) of the testicles, seminal vesicles and adrenal glands.

Statistical analysis of results was carried out by Student's «t» according to FISHER and YATES (5) and according Pearson's X^2 correlation (4).

Results

Table I shows the number per litter and weight of the neonates, and their weight at 30 days. There is no significant difference in number of neonates, but there are statistically significant differences in birth weight in the control group, with male controls weighing more than females. This intersexual difference is nonexistent in experimental animals, althoug by 30 days such a weight difference does appear in this group.

Table II shows results relative to vaginal opening, sexual cycle and percent of animals pregnated. In utero stress produces an early vaginal opening in experimental females, statistically significant with relation to controls. The same is true for the estrous cycle, wich is shorter in stressed females as a result of a shorter diestrous phase.

The percent of pregnant experimental females was 73.3 % and that of controls 66.7 %; this diference is not statistically significant. Table II also indicates the number of offspring per litter and weight at birth. These neonates are the offspring of those female whose pregnancy percentage we have just noted. There is no significant difference in offspring per litter. In regard to birth weight, the control group, but not the experimental group, displays the typical difference between females and males. This is also seen in table I.

In table III parameters of male sexual behavior are presented. It is noteworthy that the general model of sexual behavior is not altered by in utero stress. In experimental animals an increase in the number of ejaculations is seen, but it is not statistically significant when compared to controls. It is important to note, however, that ejaculatory latency (El) was always less in experimental animals, with statistically significant differences in El_1, El_2. Statistical results corresponding to the

Table I. In utero *immobilization stress: effects on number and weigt of offspring and weight to one month of age.*

	Control		Experimental			
	Female (A)	Male (B)	Female (C)	Male (D)		P
Number of pups per litter	* 4.14 ± 0.31 (7)	4.85 ± 0.66 (7)	4.00 ± 0.66 (6)	5.16 ± 0.83		N.S.
Weight of offspring	6.04 ± 0.15 (29)	6.62 ± 0.12 (33)	6.11 ± 0.20 (24)	6.45 ± 0.13 (30)	A vs B B vs C	0.01 0.05
Weight at one month	78.04 ± 2.46 (23)	84.64 ± 2.38 (28)	78.91 ± 1.71 (23)	85.33 ± 1.66 (30)	A vs B C vs D	0.05 0.05

* Mean ± S.E. In parentheses the number of animals studied. N.S. = not significant.

Table II. In utero *immobilization stress in rats: effects on vaginal opening, estrus cycle, or pregnancy and number and weight of their pups.*

	Control	Experimental	«t»	P
Vaginal opening (days)	* 38.04 ± 0.51 (21)	36.33 ± 0.24 (21)	3.03	0.01
Estrus cycle (days)	** 4.46 ± 0.09 (56)	4.19 ± 0.08 (57)	2.13	0.05
			X^2	P
Percentaje of pregnancy	66.7 %	73.3 %	0	N.S.

Number and weight of the pups

	Control		Experimental				
	Female (A)	Male (B)	Female (C)	Male (D)		«t»	P
Number	* 6.30 ± 0.40 (10)	6.10 ± 0.55 (10)	5.10 ± 0.59 (10)	6.20 ± 0.89 (10)			N.S.
Weight	5.68 ± 0.10 (63)	6.17 ± 0.12 (61)	5.80 ± 0.11 (56)	5.73 ± 0.14 (66)	A vs B	3.03	0.01
					B vs D	2.63	0.05

* Mean ± S.E. In parentheses the number of animals studied.
** Mean ± S.E. In parentheses the number of cycles studied.

fourth ejaculatory sequence no significant alteration on the parameters of the sexual behavior of male rats was shown.

In regard to male sexual behavior, we studied the percentage of valid tests of the two test carried out on each male. A valid test was defined as that in which ejaculation occurred. As can be seen in table IV, male controls performed significantly better than experimental males in the first test, but this statistical significant disappeared with the second test, in which experimental males functioned at a superior level.

Lastly table V presents the data recorded at the moment of sacrifice of the male rats. Highly significant differences are noted in all parameters: body weight of experimental animals was much less than that of controls, but the weight of seminal vesicles, adrenal glands and testicles was much greater.

Table III. In utero *Immobilization stress: Effects on the sexual behavior of male rats. First, second and third ejaculation.*

	Control	Experimental	«t»	P
NE	* 3.84 ± 0.35 (19)	4.61 ± 0.37 (21)	1.51	N.S.
Li	6.06 ± 0.93 (16)	7.27 ± 1.27 (18)	0.75	N.S.
Le_1	14.57 ± 1.70 (19)	8.50 ± 0.77 (18)	3.18	0.01
f_1	10.89 ± 1.69 (19)	7.05 ± 0.76 (18)	2.029	0.05
v_1	10.73 ± 1.32 (19)	8.70 ± 0.66 (20)	1.39	N.S.
Pr_1	5.00 ± 0.79 (19)	4.10 ± 0.36 (20)	1.04	N.S.
ANM_1	1.76 ± 0.24 (19)	1.92 ± 0.14 (21)	0.56	N.S.
Le_2	7.11 ± 0.78 (18)	4.68 ± 0.58 (19)	2.50	0.05
f_2	6.38 ± 1.21 (18)	4.89 ± 0.96 (19)	0.97	N.S.
v_2	6.61 ± 0.58 (18)	5.94 ± 0.30 (19)	1.02	N.S.
Pr_2	5.05 ± 0.38 (18)	4.42 ± 0.27 (19)	1.34	N.S.
ANM_2	2.21 ± 0.34 (18)	2.55 ± 0.20 (19)	0.87	N.S.
Le_3	5.20 ± 0.55 (15)	3.41 ± 0.20 (17)	3.15	0.01
f_3	5.33 ± 1.00 (15)	3.76 ± 0.63 (17)	1.35	N.S.
v_3	6.53 ± 0.65 (15)	5.76 ± 0.60 (17)	0.86	N.S.
Pr_3	5.26 ± 0.34 (15)	4.94 ± 0.24 (17)	0.78	N.S.
ANM_3	2.51 ± 0.25 (15)	2.69 ± 0.20 (17)	0.53	N.S.

* Mean ± S.E. Number of test in parentheses.
NE = Number of ejaculations during 60 min.
Le = Ejaculation latency (min).
v = True intromission.
ANM = Neuromotor activity.
Li = Intromission latency (min).
f = False intromission.
Pr = Refractory period.

Table IV. In utero *immobilization stress: effects on percentage of males ejaculating.*

	First test				Second test			
	Control	Experimental	X^2	P	Control	Experimental	X^2	P
Ejaculating	15	5	6.73	0.01	13	14	0.017	N.S.
No ejaculating	13	24			15	15		
Percentaje of males ejaculating	53.57	17.24			46.42	48.27		

Table V. In utero *Immobilization stress: effects on body weight and the proportionate weight of seminal vesicles, adrenal glands and testicles.***

	Control	Experimental	«t»	P
Body weight (g)	* 404.545 ± 10.52 (28)	337.689 ± 6.20 (29)	5.517	0.001
Seminal vesicles (% Bw)	0.132 ± 0.00 (28)	0.165 ± 0.00 (29)	6.032	0.001
Adrenal glands (% Bw × 10^3)	12.389 ± 0.33 (28)	16.129 ± 0.46 (29)	6.490	0.001
Testicles (% Bw)	0.798 ± 0.03 (28)	0.980 ± 0.02 (29)	4.813	0.001

* Mean ± S.E. In parentheses the number of animals studied.
** The animals were sacrified at the 4th month.

Discussion

The experimental results shown here give us a picture of the effects of immobilization stress during pregnancy on offspring. Data collected at the moment of birth (table I) indicate that these effects are not remarkable. There are not statistically significant differences in number of neonates per litter, though such differences do exist in birth weight, with male controls, as expected, heavier than females. This difference is not noted in the experimental group. However, weight at 30 days in the experimental group shows the typical dimorphism between males and females, with weights almost identical to controls. These results agree with those previously obtained (11) when cold was used as a stress agent. At four months (table V) males show a highly significant decrease in body weight, accompanied by an increase in the weight of seminal vesicles, adrenal glands and testicles.

Ordinarily, male morphology is dependent on the same androgenic stimuli which determine male behavior (13). Deficits in one are typically associated with deficits in the other (19). However, in the case of prenatally stressed male rats there is a marked dissociation between behavior and weight (20). Our results agree with these findings, since the capacity for the performance of sexual behavior tests (table IV) is enormously decreased in experimental males in the first test, while in the second the percentage of valid tests is somewhat higher in experimental than in control animals, precisely when the weight of seminal vesicles, adrenal glands and testicles is also superior to controls. This notable discrepancy in the number of valid tests seems to indicate, in effect, that prenatal stress demasculinizes sexual behavior initially (19, 21, 22) but without a profound or lasting influence, since the males recover their capacity in this respect. This is seen in table III, which give a detailed analysis of the sexual behavior model of males, and which show that there are no significant differences in this behavior.

An analysis of the effects of prenatal immobilization stress on female offspring (table II) shows that such stress produces a decrease in the estrous cycle, as well as an earlier vaginal opening, in experimental as compared to control animals. These changes appear to indicate an activation of the fertility of experimental females as a result of prenatal stress.

Under various stress conditions, sexual differentiation in some mammals is realized in the presence of large quantities of steroids, some of which are segregated by the adrenal glands (1,7). Variations in the adrenal and gonadal hormones during the period of sexual differentiation can alter reproduction in females of the litter, modifying their receptivity or gonadotrophic or ovarian secretion (7). Prenatal stress can also influence the hormonal balance between mother and fetus at the critical moment of the hypothalamic differentiation. In this regard (table II), our data conflict with that found in the literature (7), since the results of bibliography show that the prenatal stress reduces fertility and fecundity in females offspring, our results exhibit an increase in this parameters.

In addition, fertility is practically the same in our experimental and control females, and pregnancies were carried out to term. Nonetheless, it should be pointed out that these differences should be due to the stress agents used by other authors, which were severe, as opposed to our, which were weak.

Finally, we studied the offspring of those females who had suffered prenatal stress (table II). Results similar to those found in the parents (table I) were noted: there was no change in the number of neonates per litter, and the weight difference between males and females disappeared in the experimental situation.

In conclusion, though the particular stress agent studied here is a weak one, it nevertheless induces changes in male and female sexuality. This emphasizes the great influence that environment — with its many stressful situations — can have on the subsequent development of the various species, since any hormonal alteration of the mother's «milieu interieur» whether by hormonal differentiations or by means of the hypothalamic-hypophyseal-adrenal axis and activation of the sympathetic system, can lead to aberrations in the offspring.

Resumen

Se estudian los efectos de un agente estresante, la inmovilización psicofísica aplicada durante la gestación, y sus posteriores consecuencias sobre el desarrollo, la conducta y la madurez sexual de la rata.

Los resultados indican ligeras alteraciones en el desarrollo, que se acentúan a los cuatro meses de edad, momento del sacrificio. En cuanto a la conducta sexual de los machos, también se presentan leves variaciones, mientras que la madurez sexual de las hembras, medida por el momento de la apertura vaginal y el ciclo del estro, está muy modificada en los animales experimentales frente a los controles.

A la vista de los resultados, se discute la acción psicopatológica del *stress* de inmovilización in útero sobre la descendencia.

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