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BRIEF COMMUNICATION

Endotoxin Treatment of Pregnant Rats Affects Sexual Behavior of the Male Offspring

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WIJKSTRA, S., N. VALKHOF, J. M. KOOLHAAS AND G. A. SCHUILING. Endotoxin treatment of pregnant rats affects sexual behavior of the male offspring. PHYSIOL BEHAV 49(3) 647-649, 1991.—The offspring of endotoxin-infused pregnant rats (0.2 µg endotoxin, 53.3 min, day 18 of pregnancy) did not exhibit different behavior in the Hebb-Williams-type maze test, but the males showed aberrations in the sexual behavior test. Because endotoxin did not cross the placental barrier, it was concluded that the effect reflects abnormal brain development, caused by endotoxin-induced placental malfunction, notably impaired oxygen transport.

Rat Pregnancy Endotoxin Placental damage Offspring Maze test Sexual behavior

METHOD

Four-day cyclic rats of the local Wistar strain were kept in a temperature- and light-controlled room with free access to food and water (light: 0700–1900 h). Daily vaginal smears were taken. Proestrous rats were rendered pregnant by housing them at the age of 4 months with fertile males for one night (next day = day 0 of pregnancy). Rats were cannulated on day 0 of pregnancy according to Steffens (11). Endotoxin in pyrogen-free saline (E. coli, Whittaker M.A. Bioproducts Inc., Walkersville, MD) or saline alone was infused into the conscious animal under stress-free conditions on day 18 of pregnancy. Endotoxin (0.2 or 2.0 µg) was infused at the rate of 0.0375 ml/min for 53.3 min (E-rats). Control rats received the same volume of saline (S-rats). Some rats were sacrificed immediately after infusion for determination of endotoxin in maternal and fetal blood (blood of 7 to 10 fetuses was pooled) in the Limulus Amebocyt Lysate test (12). The remaining rats delivered on day 21. Pups were nursed by their own mothers. At weaning on day 21 of lactation the lighting schedule was changed to light: 2030–0830 h. At the age of 6 weeks males and females were housed separately.

Behavior tests took place at the beginning of the dark period under dimmed light conditions. At the age of 12 weeks pups were tested in a Hebb-Williams-type maze and during 10 min the frequency of rearing and crossing was registered (♀ S-rats n = 12, ♂ S-rats n = 17, ♀ E-rats n = 15, ♂ E-rats n = 14). One week
later male pups were tested in a neutral cage (80 x 55 x 50 cm).
After 5 min an estrous female was presented. During the next 10
min the males' (15 pups of S-rats and 14 pups of E-rats) behavior
was registered. Frequency and percentages of time (10 min =
100%) of the following categories of sexual behavior were
registered: investigate, mount, follow, intromission, ejaculation,
penis groom, groom and explore. For the categories mount, in-
tromission and ejaculation, latencies were recorded. If an animal
did not exhibit mount, intromission or ejaculation within the 10
min of the test, the score was considered to be 100% = 600 s.
Statistical comparison between the E and S groups were made
by the Mann-Whitney U-test. For the ejaculation response compari-
son between both groups was made by a 2 x 2 test of indepen-
dence. Statistical comparison of more than two groups was made
by analysis of variance (Kruskal-Wallis); subsequently groups
were compared in twos by the Mann-Whitney U-test (10). Differe-
ces were considered to be statistically significant when \( p < 0.05 \).

RESULTS AND DISCUSSION

In the Hebb-Williams-type maze test no differences were found
in the frequency of crossing and rearing between E- and S-rats.
Sexual behavior, on the other hand, was changed in E-rats (see
Fig. 1). With the exception of the relative time spent on groom
(an essentially nonsexual behavior; E-rats spent more time on
groom \( p < 0.05; \) Mann-Whitney U-test), no differences were
found in frequency and relative time spent on the various behavior ele-
ments between S-rats and E-rats. All S-rats (n=15) exhibited
mount, intromission and ejaculation, but only 12 out of 14 E-rats
exhibited intromission of which 5 ejaculated (\( p < 0.01; 2 \times 2 \) test
of independence). The latencies for mount, intromission and ejac-
ulation, however, were increased in E-rats (\( p < 0.05; \) Mann-Whit-
ney U-test).

After 53.3 min of endotoxin infusion of the mothers with 0 \( \mu \)g
(saline, n=4), 0.2 \( \mu \)g (n=5) or 2.0 \( \mu \)g (n=4), no endotoxin was
measured in maternal blood (detectable level 50 pg/ml), while in
the maternal blood the endotoxin concentration rose with increasing
endotoxin infusion rates: saline < 50 pg/ml; 0.2 \( \mu \)g endotoxin:
1670 ± 200 pg/ml; 2.0 \( \mu \)g endotoxin: 6800 ± 1490 pg/ml (Kruskall-
Wallis test statistic = 10.74; Mann-Whitney U-tests: \( p < 0.05 \) for
all 3 comparisons). From these data it was concluded that endo-
toxin does not cross the placental barrier and that the behavioral
effects of endotoxin treatment were not due to direct effects of
endotoxin on the fetal brain.

It is more likely that behavioral aberrations observed in this
study result from some damage inflicted to the brain of the off-
spring [cf. (5,9)] due to placental malfunction (1), notably im-
paired oxygen transport to the fetuses (13). Indeed, lack of oxygen
may result in aberrations in behavior of the offspring, e.g., maze
learning (3). Yet, because in this study pups were left with their
(endotoxin-treated) mothers, it cannot be entirely excluded that
the behavior aberrations result from disturbed maternal behavior,
since it has been shown that decreased postnatal behavior of the
mothers (licking of the pups) may result in affected sexual behav-
ior of the male offspring (8).

The endotoxin-treated pregnant rat may be a useful model for
the study of the development of the neuronal substrate underly-
ing behavioral complexes which play a role in later life. The fact
that (certain elements of) sexual behavior but not exploring
behavior is affected after treatment of rats with endotoxin on day 18
of pregnancy may suggest that the neuronal substrate underlying
sexual behavior, but not that underlying exploring behavior, is in
a critical stage of development at that time. In other words, there
may exist "critical periods" for the development of various com-
plexes of behavior. Indeed, developmental neurologists have dem-
strated that in the human there is a fixed sequence in the
appearance of behavioral patterns (4). Lack of oxygen at the on-
set of the development of the pertinent neural substrate may be a
major cause of perturbations in its function in later life.

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