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NEONATAL INHIBITION OF BRAIN ESTROGEN SYNTHESIS ALTERS ADULT NEURAL FOS RESPONSES TO MATING AND PHEROMONAL STIMULATION IN THE MALE RAT

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Abstract—Neonatal inhibition of brain estrogen formation in male rats by administration of the aromatase inhibitor, 1,4,6-androstatriene-3,17-dione (ATD), permanently changes aspects of their mating behavior and partner preference in adulthood. The medial preoptic area receives chemosensory inputs via a sexually dimorphic vomeronasal projection circuit, which responds to reproductively relevant pheromonal cues. The medial preoptic area also receives genital somatosensory inputs via the midbrain central tegmental field and the medial amygdala. We used Fos immunoreactivity as a marker of neuronal activation to determine whether there is a correspondence between the behavioral profiles of neonatally ATD-treated male rats and their neuronal responses in the medial preoptic area and other brain regions to somatosensory and chemosensory stimuli.

Achieving eight intromissions with an estrous female led to a greater neuronal Fos immunoreactivity in the medial preoptic area of neonatally ATD-treated male rats compared with neonatally cholesterol-treated male rats. Exposure for 1.5 h to chemosensory cues derived from soiled bedding of estrous females induced Fos immunoreactivity throughout the vomeronasal pathway (i.e. medial amygdala, bed nucleus of the stria terminalis and medial preoptic area) in both ATD and cholesterol males (Experiment 2a). By contrast, exposure for 1.5 h to chemosensory cues derived from soiled bedding of sexually active males revealed clear differences between ATD and cholesterol males in neuronal Fos immunoreactive (Experiment 2b). At peripheral portions of the vomeronasal pathway (i.e. the accessory olfactory bulb and the medial amygdala), there were no differences in the number of Fos-immunoreactivity neurons between ATD and cholesterol males. However, neurons in the more central portions of the vomeronasal pathway (i.e. the bed nucleus of the stria terminalis and the medial preoptic area) showed increased Fos immunoreactivity after exposure to odors from sexually active males in ATD males as opposed to cholesterol males. Females, like ATD males, showed neuronal Fos immunoreactivity at each level of the vomeronasal pathway after being exposed to odors from sexually active males.

These results suggest that the responsiveness of neurons in the central portion of the vomeronasal projection circuit to odors from sexually active males, but not estrous females, is sexually differentiated in male rats due to the neonatal action of estrogens. Copyright © 1996 IBRO. Published by Elsevier Science Ltd.

Key words: neonatal ATD treatment, Fos immunoreactivity, sexual behavior, pheromonal stimulation, preoptic area.

Perinatal administration of male rats of 1,4,6-androstatriene-3,17-dione (ATD), which blocks the aromatization of testosterone to estradiol, affects the sexual differentiation of the CNS, as well as psychosexual differentiation.[2,7,10,21,32,33] When tested in adulthood while gonadally intact, ATD-treated males show an altered sexual partner preference. When given free access to an estrous female and a sexually active male, they will approach, mount and intromit with the estrous female. However, they will also approach the active male, to whom they show proceptive behaviors (presenting, earwiggling, hopping and darting) and lordosis when mounted.[2–4,7] Control males, treated perinatally with cholesterol, prefer to mate with the estrous female and rarely approach the sexually active male. The most robust differences in partner preference between neonatally ATD-treated male and normal male rats were obtained when subjects were castrated as adults and subsequently treated with estradiol: ATD males showed a clear-cut

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Abbreviations: AOB, accessory olfactory bulb; ATD, 1,4,6-androstatriene-3,17-dione; BNST, bed nucleus of the stria terminalis; CTF, central tegmental field; IR, immunoreactive, immunoreactivity; MePD, posterodorsal portion of the medial amygdala; MePV, posteroventral portion of the medial amygdala; mPOA, medial preoptic area; PBS, phosphate-buffered saline; POA, preoptic area; SDN, sexually dimorphic nucleus.

preference for the sexually active male, whereas normal males preferred the estrous female.[3]

Numerous studies[30] have shown that bilateral lesions of the medial preoptic area (mPOA) anterior hypothalamus continuum disrupt masculine coital function in male vertebrates and enhance males' proceptive and receptive responsiveness towards a stimulus male in cats,[19] rats[20,24,26] and guinea-pigs,[29] and induces a partner preference for a male in ferrets.[27] Within the mPOA, a sexually dimorphic nucleus (SDN-POA), which is significantly larger in males than in females, was first described in rats.[17] In rats, the sexual differentiation of the SDN-POA shows a close parallel with psychosexual differentiation, i.e. its larger size in males depends on the action of estrogenic metabolites of testosterone during a critical perinatal period.[14,15,22] This correlation was emphasized by a recent study in which SDN-POA volume was reduced in both prenatally and pre- and neonatally ATD-treated males, with a larger reduction being achieved in the latter than in the former group.[21]

Immunocytochemical visualization of Fos, the nuclear protein product of the immediate early gene, *c-fos*, has provided useful information about the neural circuits which are activated following mating in male rats.[5] In male rats, neural Fos immunoreactivity (IR) is increased after mating in several forebrain regions, including the mPOA, bed nucleus of the stria terminalis (BNST) and posterodorsal portion of the medial amygdala (MePD), and in the midbrain central tegmental field (CTF).[5,6,28,34] We used Fos-IR as a marker of neural activity to reveal possible differences between neonatally estrogen-deprived (i.e. neonatally ATD-treated) males and normal (i.e. neonatally cholesterol-treated) male rats' responses to genital and pheromonal stimulation associated with sexual behavior. In Experiment 1, the number of Fos-IR neurons was compared in several brain regions of adult male rats treated neonatally with ATD or cholesterol. Animals were killed 1 h after achieving eight intromissions with an estrous female.

In rodent species, reproductivity relevant pheromonal cues are detected by receptors in the vomeronasal organ, which in turn transmit this information centrally via the accessory olfactory bulb (AOB), the medial amygdala, BNST and mPOA.[23] In the rat, this vomeronasal projection pathway is sexually dimorphic, with males having more vomeronasal receptors and a larger number of neurons throughout.[31] In male rats, the sexual differentiation of this vomeronasal pathway apparently occurs under the influence of perinatal estrogens.[18,31] Therefore, in Experiment 2, we investigated whether pheromonal cues (derived from either the urine and feces of estrous females or sexually active males) may reveal differences in neural *c-fos* responses in the vomeronasal projection to the mPOA and/or the nucleus accumbens, a brain region which has been linked to reward and sexual motivation,[16,25,35] between neonatally ATD-treated males and cholesterol-treated males.

EXPERIMENTAL PROCEDURES

Animals

Male and female Wistar RP rats bred in our laboratory were housed in single-sex groups of two to three. Food and water were available *ad libitum*. All rats were kept on a reversed 14:10 h light–dark cycle (lights off from 7.45 a.m. to 5.45 p.m.). Female rats were time-mated and parturition occurred 22 days later. Within 2–4 h after birth, the newborn males received subcutaneously a silastic capsule (SR3: inner diameter 1.5 mm, outer diameter 2.1 mm, length 5 mm) containing crystalline ATD or cholesterol under ice anesthesia. The implants were removed at 21 days of age (day of weaning), and the animals were housed two to three of the same treatment to a cage.

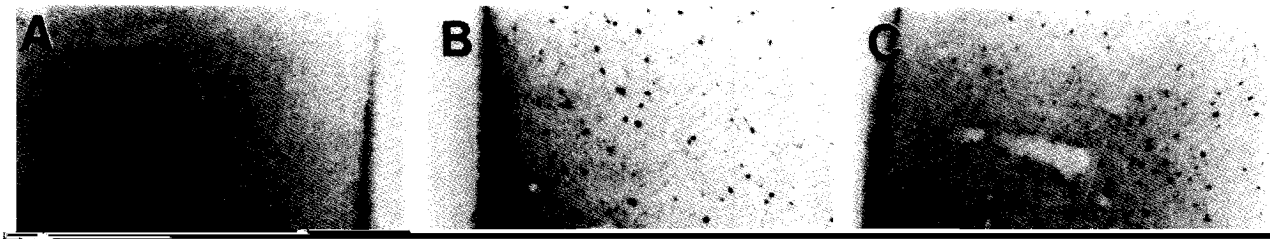
Experiment 1: *neural* c-fos *responses to mating*

At the age of approximately three months, 15 neonatally ATD-treated and 15 cholesterol-treated males were castrated under ether anesthesia and were injected daily with testosterone propionate (200 μg, s.c., in olive oil). The ATD and cholesterol males were pretested three times (30 min/test) with an estrous female in order to provide them with heterosexual experience. All behavioral testing was conducted in semicircular cages measuring $62 \times 40 \times 36$ cm.^{3} Before testing, the experimental animal spent a 15-min adaptation period in the cage. The test began when a female was put in the cage. The stimulus females were ovariectomized and brought into behavioral estrus by injecting 20 μg of estradiol benzoate 24–48 h followed by 1.0 mg of progesterone 3–4 h before testing.

All males were housed singly for one or two days prior to the actual experiment. Groups of ATD and cholesterol males were allowed to achieve eight intromissions with an estrous female. Then, these males were put singly in their home cages for 1 h, after which they were perfused and their brains processed for Fos-IR. Pairs of ATD and cholesterol males were matched for number of mounts and intromissions, and testing time. Additional male rats (ATD and cholesterol; housed singly for one or two days) taken directly from their home cages served as unmated controls. In Experiment 1, 10 mated ATD, 10 mated cholesterol, five unmated ATD and five unmated cholesterol males were used.

Experiment 2*a*: *neural* c-fos *responses to chemosensory cues derived from soiled bedding of estrous females*

At the age of approximately six months, five neonatally ATD-treated and 10 cholesterol-treated males were castrated under ether anesthesia using a midline abdominal incision and received estradiol subcutaneously through a silastic capsule (inner diameter 0.5 mm, outer diameter 1.0 mm, length 2.5 cm). We chose adult estradiol treatment because the sexual behavior of ATD males differed most dramatically from that of control males under this hormonal condition.[3] Two groups of ovariectomized females (six per group) were injected subcutaneously with estradiol benzoate (20 μg) 24–48 h prior to a progesterone (1.0 mg) injection. Three hours later, all females were placed in clean cages which contained fresh sawdust. Bedding was collected 12 h later and immediately used in the experiment. The heterosexually experienced ATD and cholesterol males were housed singly for one to two days prior to use in the experiment in a room containing no other rats. Pairs of ATD and cholesterol males were perfused after spending 1.5 h in the cage containing soiled bedding from estrous females and their brains were processed for Fos-IR. Additional male rats, housed singly in a separate room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