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CENTRAL TEGMENTAL FIELD AND SEXUAL BEHAVIOR IN THE MALE RAT: EFFECTS OF NEUROTOXIC LESIONS

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Abstract—The medial preoptic area/anterior hypothalamus (MPOA/AH) is a key structure in the control of male sexual behavior. This area has reciprocal connections with mesencephalic and brainstem structures including the central tegmental field (CTF). It has been suggested that the CTF receives somatosensory information generated in the genitals promoting activation of the MPOA/AH. In the present study we evaluated the effects of bilateral neurotoxic lesions of the CTF upon male rat sexual behavior. We also explored the effects of these lesions on sociosexual behaviors, partner preference, sexual incentive motivation and motor execution. Tests were performed before and after bilateral quinolinic acid infusions. The lesion was evaluated by quantifying neuronal nuclei (Neu-N) and by the presence of glial fibrillary acidic protein (GFAP) immunohistochemistries. A significant reduction in the percentage of animals displaying mounts, intromissions, and ejaculations was observed in the bilateral and misplaced lesion groups 1 week after the lesion. In the second week post-lesion, only animals with bilateral damage of the CTF showed a significant reduction in sexual behavior. In the third post-lesion test, the percentage of animals displaying sexual behavior returned to control levels. The frequency of pursuit and self-grooming was reduced, and genital exploration was increased after the lesion. Partner preference and sexual incentive motivation were not affected by the lesion suggesting that the CTF is not involved in the appetitive aspects of sexual behavior. Mount, intromission, and ejaculation latency were increased in animals with damage of the CTF and in animals with lesions outside this region. Motor execution was also affected in both groups, suggesting that alterations in latencies could be associated with damage not specific to the CTF.

Key words: male sexual behavior, neurotoxic lesion, sociosexual behavior, partner preference, sexual incentive motivation, motor execution.

Lesions of the medial preoptic area/anterior hypothalamus (MPOA/AH) virtually eliminate male sexual behavior in all species studied (Meisel and Sachs, 1994; Paredes, 2003). In the rat, electrolytic (Heimer and Larsson, 1966, 1967; Paredes et al., 1993, 1998) as well as neurotoxic (Hansen, 1982) lesions of the MPOA/AH permanently abolish mating. Associated with the inhibition of male sexual behavior, MPOA/AH lesions reduce sociosexual behaviors displayed during mating, specifically frequency and duration of pursuit and anogenital exploration. This has been interpreted as a reduction in sexual motivation (Paredes et al., 1993; Agmo, 1999). Further support for this proposal is derived from studies evaluating partner preference. Male rats (Paredes et al., 1998) and ferrets (Kindon et al., 1996) change their preference after lesions of the MPOA/AH. Before the lesion, males of both species showed a clear preference for a sexually receptive female, but after the lesion they prefer a sexually experienced male. In the same way, Edwards and Einhorn (1986) showed that male rats with MPOA/AH lesions spent the same time with a sexually receptive female and a non-receptive female, whereas before the lesion, they spent significantly more time with the receptive female. These results suggest that MPOA/AH lesions reduce sexual motivation (Paredes and Baum, 1995; Paredes, 2003 for a review).

Reciprocal connections between the MPOA/AH and caudal brain structures have been identified by autoradiography and tracer studies (Coolen et al., 1998; Conrad and Pfaff, 1976; Simerly and Swanson, 1986). The MPOA/AH sends efferents to the central tegmental field (CTF). A dense projection from the CTF to the MPOA/AH has been described as well (Simerly and Swanson, 1986). The CTF may have a role in male sexual behavior. For example, an increase in the expression of the protein Fos, a product of the immediate early gene *c-fos* that reflects neuronal activation, is observed in this region after mating but not after exposure to sexually relevant olfactory cues (Baum and Everitt, 1992; Coolen et al., 1996; Greco et al., 1998). Interestingly, the combined lesion of the CTF and ipsilateral medial amygdala significantly reduces Fos expression in the ipsilateral MPOA/AH after mating, while lesions of either one of these structures alone do not affect Fos expression in the MPOA/AH (Baum and Everitt, 1992). The authors interpreted these results as suggesting that the medial amygdala promotes neuronal activity in the MPOA/AH due to olfactory-vomeronasal activation, while the CTF promotes activation of the MPOA/AH as a consequence of genital somatosensory activation (Baum and Everitt, 1992). It has also been demonstrated that male rats with bilateral transection of the pelvic nerves showed reduced expression of Fos in the CTF after mating (Wersinger et al., 1993), suggesting that the activation of the CTF after mating is due to somato-

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Abbreviations: ANOVA, analysis of variance; CTF, central tegmental field; DAB, diaminobenzidine; DLT, dorsolateral tegmentum; GFAP, glial fibrillary acidic protein; MPOA/AH, medial preoptic area/anterior hypothalamus; Neu-N, neuronal nuclei; PBS, phosphate buffer saline; r.p.m, revolutions per minute; SPFp, subparafascicular thalamic nucleus.

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sensory stimulation. Neurons of this region that express Fos after mating colocalized with androgen receptors; thus, androgen sensitive neurons of the CTF may be activated during the expression of sexual behavior (Greco et al., 1998). Although different lines of evidence suggest a participation of the CTF in the control of male sexual behavior, no study has yet evaluated the effects of lesions of this brain area upon sexual behavior.

The main goal of the present study was to determine the effect of a bilateral neurotoxic lesion of the CTF on sexual behavior. We also tested the effects of the lesions on sociosexual behavior, partner preference, sexual incentive motivation, and motor execution. This would give us detailed information as to the role of the CTF in male sexual behavior.

EXPERIMENTAL PROCEDURES

Animals

Thirty male Wistar rats (250–300 g) from a local colony were used. They were sexually naive at the beginning of the experiment. Only males that ejaculated with receptive females in three tests of sexual behavior were used in the study. Wistar female rats (200 g) bilaterally ovariectomized were used as stimuli. They were treated with 25 μg of estradiol benzoate (48 h) and 1 mg of progesterone (4 h) before observation. The synthetic hormones were bought from Sigma Company (St. Louis, MO, USA) and were dissolved in corn oil. Males and females were maintained under a reversed 12-h light/dark cycle. Food (Laboratory Rodent Diet 5001; PMI Inc., St. Louis, MO, USA) and water were always available. The experiments were carried out in accordance with the "Reglamento de la Ley General de Salud en Materia de Investigación para la Salud" of the Mexican Health Ministry, and protocols were approved by the Institute Animal Care Committee. This committee follows National Institute of Health (NIH; USA) guidelines including minimizing the number of animals for the experiment and taking measures to minimize their suffering.

Behavioral testing procedure

Male sexual behavior tests. Sexual behavior was registered in a rectangular arena (40 cm×50 cm×30 cm) where a receptive female had already been placed. Subjects were tested once a week for three consecutive weeks. Conventional sexual behavior variables were recorded: latency to the first mount, intromission and ejaculation, the number of mounts and intromissions, as well as the post-ejaculatory interval. The test was ended after 60 min if no mounts or intromission were observed. Only males that ejaculated in all three pre-lesion sessions were included in the study.

Sociosexual behavior tests. The following behaviors were evaluated during the first 10 min of the third pre-lesion and third post-lesion test: pursuit (the male follows the female keeping close contact); grooming partner (licking or biting the partner); genital exploration (sniffing or biting the female genitals); self-grooming (licking or biting of the fur: limbs or genital region); rearing (standing on the hind legs); sniffing (rapid movement of the head or the whiskers while the animal explores the environment or a particular object like the sawdust or part of the cage); and resting (lying or standing still). The software observer (Noldus 3) was employed to quantify the frequency of sociosexual behaviors.

Partner preference test. Partner preference was evaluated in a three-compartment box made of wood. The middle compartment communicated with the lateral compartments through a 10×10-cm sliding door on each side. A sexually active stimulus male was placed in one of the lateral compartments, and a sexually receptive female was placed in the opposite lateral compartment. The stimulus animals wore a harness attached to the rear of the compartment with flexible rope. In this way, they were able to freely display coital behavior but had a limited action radius within their compartment. The preference score was calculated as the amount of time spent with the receptive female divided by the amount of time spent with the receptive female plus the amount of time spent with the sexually active male. A score of 0.5 indicates no preference; a higher score indicates preference for the receptive female and a lower score a preference for the male. Subjects were tested for their preference to interact with the stimulus animals in four independent 10-min tests, twice before and twice after the lesion. These tests were done 3 days after the first sexual behavior test and 3 days after the second sexual behavior test. Two partner preference tests after the lesion were done following the same schedule used as in the pre-lesion tests.

Sexual incentive motivation. We used a standardized test similar to the one described by Agmo (2003). Briefly, the test is performed in a black arena (100×50×45 cm) with two openings (25×25 cm) diagonally opposed on the long walls and covered with wire mesh. On each opening a black cage (25×25×15 cm) containing a receptive female or a sexually active male were placed. In this way, the experimental subjects can see, smell, and hear the incentive stimulus. Inside the arena and adjacent to each incentive animal cage, a virtual area (30×20) named incentive zone was defined. The test started immediately when an experimental subject was placed in the arena. The time in the incentive zone and the distance moved during 10 min were evaluated using a video track system (Ethovision Pro, Noldus, Wageningen, The Netherlands). Before the test, the experimental subjects were habituated to the sexual incentive motivation arena with the stimulus cages empty during three sessions of 10 min each done on three consecutive days. Data were expressed as preference score, as in the partner preference test. In this way a score higher than 0.5 indicates a higher sexual incentive motivation for the female.

Motor execution. A rota-rod apparatus (Accuscan Electronics, Columbus, OH, USA) that consist of a cylinder (3.5 inches in diameter) rotating at different speeds was used. Subjects were trained on the rota-rod with a speed increasing from 0 to 30 revolutions per minute (r.p.m.) in a session lasting 180 s. The males were trained once in the morning and once in the afternoon on four consecutive days. The test was performed using a speed of 35 r.p.m. for 180 s and counting the number of falls. The pre-lesion and post-lesion motor execution tests were done after the second sexual behavior test, respectively.

All behavioral tests were performed within 3 weeks. Sexual behavior was evaluated on days 1, 8, and 15. Sociosexual interactions were recorded on the last sexual behavior test (day 15). Partner preference was evaluated on days 5 and 12. Animals were habituated for sexual incentive motivation on days 16, 17 and 18 with the test performed on day 19. Subjects were trained for motor execution on days 9–12 and tested on day 13. Starting 1 week after the lesion, the same sequence of behavioral tests was repeated.

Surgery

The animals were deeply anesthetized with pentobarbital (35 mg/kg) and placed in a Kopf stereotaxic instrument. The lesion was produced by injecting the *N*-methyl-D-aspartate receptor agonist quinolinic acid (0.12 M in phosphate buffer saline (PBS); pH 7.4) in 20 subjects. Ten animals were injected with vehicle solution. The neurotoxic solution was freshly prepared before use and kept