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Intracranial Cycloheximide: Effect on Male Mouse Sexual Behavior and Plasma Testosterone[1]

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QUADAGNO, D. M., S. M. ALBELDA, T. E. MCGILL AND L. J. KAPLAN. *Intracranial cycloheximide: effect on male mouse sexual behavior and plasma testosterone.* PHARMAC. BIOCHEM. BEHAV. 4(2) 185–189, 1976. – Cycloheximide (Cyclo), an inhibitor of protein synthesis, infused bilaterally into the preoptic area (POA) of intact B6D2F$_1$ male mice significantly inhibited male sexual behavior when the males were presented with receptive females 12 hr after treatment. The few males that ejaculated appeared to copulate normally. This finding suggests that Cyclo acts primarily by inhibiting sexual arousal rather than sexual performance. The inhibition of sexual behavior was not observed when the males were tested 84 hr after treatment. After exposure to an estrous female, plasma testosterone levels were measured in males with POA infusions of Cyclo or saline vehicle. No significant difference was found, but both groups had significantly higher levels of plasma testosterone than males not exposed to estrous females. It is suggested that the interference with sexual behavior by Cyclo was not due to interference with the neuroendocrine mechanisms controlling blood andorgen levels, but due to Cyclo acting directly on the neural circuits controlling sexual responsiveness.

Sexual behavior Male mouse Cycloheximide Plasma testosterone

CYCLOHEXIMIDE (Cyclo) is an inhibitor of protein synthesis by direct action at the ribsomal level [18]. This compound has recently been used to study the role of various brain regions in the mediation of specific behaviors. For example, the inhibitory action of progesterone on estrous behavior was blocked in the female guinea pig by the infusion of Cyclo into the ventromedial hypothalamus [19]. Preoptic area (POA) infusions of Cyclo have been shown to inhibit reversibly maternal behavior and steroid-induced sexual receptivity in the female rat [9,17].

The POA has also been implicated in the control of male sexual behavior [5]. Testosterone propionate implants into this region caused a restoration of sexual behavior in castrated male rats [4,10] while complete lesions of the POA abolished the display of male sexual behavior in intact rats [2, 7, 11].

The effects of Cyclo infusions in the POA of males has not previously been studied. The purpose of the present experiment was to study the effects of such infusions on sexual behavior and plasma testosterone (T) levels in intact male mice.

METHOD

Experiment 1

Animals. Male B6D2F$_1$ mice, resulting from a cross between C57BL/6J females and DBA/2J males, were used.

Procedure. Litters were weaned at 28 days of age. The males were housed with their brothers from weaning until isolation in individual cages at approximately 8 weeks of age. The entire colony was maintained on a 12:12 reversed light/dark cycle.

At approximately 10 weeks of age behavioral testing was begun. All testing occurred under normal room illumination during the middle of the dark phase of the cycle. The test animals were presented with stimulus females of the

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BALB/cJ strain brought into behavioral estrus by injections of 0.01 mg of aqueous estradiol in 0.02 ml distilled water 24 hours before testing.

A test session began when an estrous female was introduced into the home cage of the test male. The lid of the test male's cage containing food and water was replaced by a clear flat plastic lid after introduction of the estrous female. The test male was observed while with the stimulus female for 1 hr.

Sexual behavior of the male house mouse consists of a series of mounts and intromissions. A mount may be considered an unsuccessful attempt to penetrate the female while an intromission is a successful penetration. When the male mounts, he palpates the female's sides with his forepaws and executes a series of very rapid, probing, pelvic thrusts. If he gains intromission, the rate of thrusting is reduced to about 2 per sec. A typical intromission consists of approximately 25 thrusts. After a series of about 20 intromissions the ejaculatory reflex (ER) occurs. As a male mouse approaches ejaculation, the speed of thrusting is increased; the male then quivers strongly, clutches the female with all four limbs, and falls to his side after ejaculation [13].

The following behavioral parameters were recorded during the 1 hr observation period: (1) mount latency (ML) time from introduction of the female to the first mount, with or without intromission, by the male, (2) intromission latency (IL) – time from introduction of the female to the first intromission, and (3) ejaculation latency (EL) – time from the first intromission to the beginning of the ejaculatory reflex (ER). The numbers of mounts and intromissions were also recorded. If ejaculation did not occur during the 1 hr test period, the stimulus female was left overnight with the male, and the following morning the stimulus female's vagina was examined for a copulatory plug. The presence of a copulatory plug indicated that an ER had occurred during the night.

The males were tested twice weekly with a minimum of 72 hr between ERs. When a male exhibited 2 consecutive ejaculatory responses (either direct observation of an ER or the presence of a vaginal plus in the stimulus female), he was selected for experimental treatment. After the second consecutive ER, the animal was given the usual 72 hr period before the next sex test. However, 12 hr before the next test the males were randomly assigned to 1 of 4 treatment groups: (1) bilateral preoptic area (POA) infusions of 50 μg Cyclo/1 μl saline per side (N = 10 successful placements of 13 animals infused); (2) bilateral POA infusions of 1 μl of saline solution (N = 15 successful placements of 17 animals infused); (3) bilateral caudate nucleus (CN) infusions of 50 μg Cyclo/1 μl saline per side (N = 4 successful placements of 4 infused); and (4) 100 μg Cyclo/2 μl saline subcutaneous under the nape of the neck (N = 4). Thus, after 2 pretreatment tests those animals exhibiting 2 consecutive ERs were treated with saline or Cyclo.

Twelve hr after Cyclo or saline treatment, the animals were again tested for male sexual behavior as previously described. At the end of 1 hr, direct observation was terminated, but the test animal and stimulus animal were left together for 6 more hr. At the end of that time, the stimulus female was removed and her vagina examined for the presence of a copulatory plug. The test was terminated at this point since it has been shown that the effect of Cyclo is inhibiting steroid-induced sexual receptivity in female rats is reversible after 24–36 hr [17].

The test animal was rested for another 72 hr and retested. This was repeated so that the test animal had 3 sex tests after the Cyclo or saline treatment, 1 after 12 hr, 1 after 84 hr, and 1 after 156 hr. On the first sex test after treatment the stimulus female was only left in with the male for a total of 7 hr, but on the last 2 tests the female was left with the male overnight. When the female was removed, her vagina was examined for a copulatory plug.

Cyclo was dissolved in a vehicle consisting of 9 parts saline (0.85% NaCl) and one part India ink marker. The final concentration was 50 μg Cyclo per μl of solution. Stereotaxic surgery, while the animals were under ether anesthesia, was used and the infusions were done with a microliter syringe. When the desired brain location was reached the Cyclo or saline solution was injected and the blunt-end needle (28 ga) left in place for 30 sec before it was removed from the brain. The stereotaxic coordinates for the POA and caudate nucleus were modified from Montemurro and Dukelow [16] and Cook [3]. The boundaries of the POA were defined, relative to bregma, as 0.00 anterior-posterior plane; 0.05 mm lateral from midline and 4.6 mm ventral to the cortex. The boundaries of the caudate nucleus were defined, relative to bregma, as 0.00 anterior-posterior plane, 2.5 mm lateral from midline and 3.0 mm ventral to the cortex.

Immediately following the third behavioral test after experimental treatment, the test animals were perfused through the heart with physiological saline followed by 10% Formalin. The brains were removed and fixed in 10% Formalin for at least 48 hr. The brains were then sectioned at 50 μ intervals while frozen and stained with cresyl violet. Only data from animals with infusions in the correct brain areas were utilized in the present study.

Experiment 2

In an attempt to elucidate the mechanism of action of Cyclo, plasma testosterone levels were examined after test males were exposed to receptive females.

Animals. Male B6D2F$_1$ mice of approximately 20 weeks of age were used.

Procedure. Mice were weaned, housed and fed in the same manner as the subjects in Experiment 1. Each male selected for the study had shown 4 ERs (as evidenced by copulatory plugs). The mice were randomly divided into 2 groups. One group received saline into the POA (N = 16 successful placements of 18 animals infused) and the other group received Cyclo into the POA (N = 10 successful placements of 18 animals infused). The animals were anesthetized and the appropriate infusion was made in the same manner as in Experiment 1. After an overnight recovery period (12–18 hr after infusion), a wire mesh was placed across the center of the animal's cage and an estrous female (BALB/CJ strain brought into behavioral estrus by injections of 0.01 mg estradiol 24 hr before the test) was placed on the opposite side of the divider. The wire barrier allowed visual, auditory, and olfactory communication between the mice, but prevented any actual physical contact. After 30 min of exposure, the animal was removed from the cage and a blood sample (approximately 0.2 ml) was obtained from the unanesthetized animal by puncturing the opthalamic venous plexus. The serum was removed and frozen until the assay was performed. After bleeding, the mice were killed and the brains fixed in Formalin. The brains were subsequently frozen, sectioned

TABLE 1

INFLUENCE OF CONTROL PROCEDURES AND INFUSIONS OF CYCLOHEXIMIDE (CYCLO) INTO THE PREOPTIC AREA (POA) ON MALE SEXUAL BEHAVIOR IN THE INTACT MALE MOUSE

Group	+ 12 hr Posttreatment			+ 84 hr Posttreatment			+ 156 hr Posttreatment		
	% MT	% INTRO	% ER	% MT	% INTRO	% ER	% MT	% INTRO	% ER
Saline-POA (N = 15)	100	100	73 (60)	100	100	80 (66)	100	100	100 (80)
Cyclo-SC (N = 4)	100	100	100 (75)	100	100	100 (100)	100	100	100 (100)
Cyclo-CN (N = 4)	100	100	100 (100)	100	100	100 (100)	100	100	100 (100)
Cyclo-POA (N = 10)	40*	40†	20‡ (20)	100	100	80§ (70)	100	100	90§ (80)

Ejaculation was measured as the percentage of animals to ejaculate during the 1 hr observation period; or if ejaculation did not occur during the observation period ejaculation was also measured as the percentage of stimulus females that showed a copulatory plug after being housed overnight with the test male. The numbers in parentheses indicate the percentage showing the ejaculatory response (ER) in the presence of the observer during the 1 hr test period. The percentage of animals to mount (MT) without intromission and to intromit (INTRO) during the 1 hr observation period is also shown. SC–subcutaneous, CN–caudate nucleus.

*Significantly different from Saline POA at 12 hr test ($\chi^2 = 11.84$, $p<0.001$)

†Significantly different from Saline POA at 12 hr test ($\chi^2 = 11.84$, $p<0.001$)

‡Significantly different from Saline POA at 12 hr test ($\chi^2 = 6.83$, $p<0.01$)

§Significantly different from Cyclo POA at 12 hr test ($\chi^2 = 5.05$, $p<0.05$)

at 50 μ, and stained with cresyl violet. Only data from animals with infusions in the correct brain areas were utilized in the present study.

The serum was extracted with ether. The ether extracts were assayed without chromatography because circulating levels of the only interfering steroid (dihydrotestosterone) are very low in the mouse [6]. The radioimmunoassay procedure of Lucas and Abraham was used [12]. The values of the unknown samples were calculated by interpolation from a standard curve after corrections for recoveries and portion size were included. Sensitivity of the assay was 5 pg. Precision, defined as the coefficient of variation for duplicate determinations, was 7.7% for inter-assayed samples and 10.9% for intra-assayed samples. Buffer blank values averaged 0.1 ng/ml.

RESULTS

Experiment 1

The effect of POA infusions of Cyclo and of control procedures on the percent from each group to mount, intromit, and ejaculate is shown in Table 1. The data were analyzed using the Chi square test. Twelve hr after POA infusions of Cyclo significantly fewer males mounted, intromitted, and ejaculated when compared to the males receiving POA infusions of saline or the other control groups (Table 1). During the testing period the males receiving POA infusions of Cyclo explored the cage, sniffed the genital region of the stimulus female, but generally did not initiate mounting or intromission behavior. The animals all appeared in excellent health at the time of testing. At 84 and 156 hr after treatment all groups showed comparable levels of mounting, intromitting, and ejaculation. There were no significant differences between any of the groups at these times.

The effect of POA infusions of Cyclo and of control procedures on the latencies to first mount, intromission, and ejaculation, plus frequency of mounts and intromissions, was examined by analysis of variance followed by post hoc analysis with the Student-Newman-Keuls test. No significant differences existed between any of the groups 12 hr after treatment. The 2 animals receiving POA infusions of Cyclo that showed the entire sequence of mating behavior, i.e., mounting, intromission, and ejaculation showed latencies and frequencies within the range set by the controls. When the same statistical tests were applied to the sexual behavior data measured 84 and 156 hr after treatment there again were no significant differences between any of the groups.

Experiment 2

The influence of POA infusions of Cyclo or saline on serum testosterone levels in male mice exposed to receptive females is shown in Table 2. Although the mean testosterone level for the saline group was slightly higher than that of the Cyclo group, the difference was not significant when analyzed with the Mann-Whitney U-test (U = 104).

Also shown in Table 2 are the results for a separate group of 14 males that were not exposed to receptive

TABLE 2

SERUM TESTOSTERONE LEVELS OF CONTROL AND HYPOTHAMICALLY INFUSED MALE B6D2F$_1$ MICE

Group	Treatment	No. of Animals	Testosterone Levels (ng/ml)
Cyclo-POA	1/2 hr non-contact sexual exposure	10	14.7 ± 2.3 (SEM)
Saline-POA	1/2 hr non-contact sexual exposure	16	17.4 ± 2.8 (SEM)
Control	none	14	4.1 ± 0.3 (SEM)

females, but bled immediately after removal from the home cage.

Both experimental groups in Table 2 had significantly higher plasma testosterone values than the males not exposed to estrous females (Mann-Whitney U = 18, $p<0.02$ for Cyclo group versus non-exposed group and U = 32, $p<0.002$ for saline group versus non-exposed group). Thus it would appear that the interference in sexual responsiveness seen in Experiment 1 was not due to interference with the neuroendocrine reflexes controlling blood androgen level.

DISCUSSION

The data presented in this report demonstrate that Cyclo, an inhibitor of protein synthesis, infused into the POA of male mice disrupts sexual responsiveness. Unlike lesions of the POA which permanently abolish male sexual behavior [2, 7, 11], Cyclo's effect was totally reversible with complete restoration of mating behavior 84 hr after treatment. These findings are in agreement with previous reports that Cyclo in the POA of female rats reversibly blocks both maternal and sexual behavior [9,17].

Not all of our Cyclo-treated males showed complete abolition of sexual behavior. Four of 10 males mounted and intromitted 12 hr after infusion. Two of these achieved ejaculations in what appeared to be normal matings, i.e., their behavioral measures were within the range set by control groups. It is possible that these two animals received sub-threshold doses of Cyclo; although the histological marker indicated infusion into the POA, some of the substance may have returned up the cannula tract. The dose of Cyclo has been shown to be critical for the inhibition of steroid-induced sexual receptivity in the female rat [8].

While the data base is limited, it may be useful to put these results into a theoretical framework. The most influential theory of rodent sexual behavior is the dual-mechanism proposal of Beach [1]. The theory postulates an arousal mechanism (AM) that is responsible for the initiation of sexual behavior (measured by ML and IL), and a copulatory mechanism (CM) that controls the mating pattern from the first intromission to ejaculation. The CM is measured by such variables as EL and number of intromissions.

Our results indicate that Cyclo-induced inhibition of protein synthesis in the POA affects the AM in at least one and perhaps two ways. Sixty percent of POA Cyclo infused males did not mount receptive females 12 hr after treatment. Thus one effect is complete suppression of the AM. Of the 4 males that did initiate a mating sequence, 2 ceased copulating prior to the ER even though their MLs and ILs were within the normal range. Incomplete mating may represent a second effect of POA Cyclo on the AM, perhaps produced by a dose level lower than that required for complete suppression of the AM.

Obviously the effect of POA Cyclo on the CM cannot be determined for those males in which complete suppression of the AM occurred. The CM of the 2 POA Cyclo males that ejaculated appeared normal, but it is possible that particular dose levels may affect this mechanism.

Considering the major effect of POA Cyclo in the present experiment (complete suppression of the AM), there are several possible physiological pathways of Cyclo action. While the treatment might have affected hormone levels and thus acted to inhibit sexual behavior, our data would appear to eliminate this possibility since there was no significant difference in the plasma testosterone levels of Cyclo or saline treated mice; both groups showed the characteristic testosterone surge after 30 min of non-contact sexual exposure to a receptive female. It appears, then, that Cyclo acts directly on the sensory, motor, or associative functions of those neurons involved in sexual arousal.

REFERENCES

1. Beach, F. A. Characteristics of masculine sex drive. In: *Nebraska Symposium on Motivation*, edited by M. R. Jones. Lincoln: University of Nebraska Press, 1956, pp. 1–32.
2. Chen, J. J. and D. K. Bliss. Effects of sequential preoptic and mammillary lesions on male rat sexual behavior. *J. comp. physiol. Psychol.* 87: 841–847, 1974.
3. Cook, R. The effect of electrolytic lesions on ingestive, emotional, and sexual behaviors in the B6D2F$_1$ mouse. Unpublished Senior Honors Thesis, Williams College. 1973.
4. Davidson, J. M. Activation of the male rats sexual behavior by intracerebral implantation of androgen. *Endocrinology* 79: 783–788, 1966.

5. Davidson, J. M. Hormones and reproductive behavior. In: *Hormones and Behavior*, edited by S. Levine. New York: Academic Press, 1972, pp. 64–98.
6. Falvo, R. E. and A. V. Nalbandov. Radioimmunoassay of peripheral testosterone in males from eight species using specific antibody without chromatography. *Endocrinology* **95**: 1466–1470, 1974.
7. Heimer, L. and K. Larsson. Impariment of mating behavior in male rats following lesions in the preoptic-anterior hypothalamic continuum. *Brain Res.* **3**: 248–263, 1966/1967.
8. Ho, G. The effect of intracranial cycloheximide on sexual behavior, maternal behavior and ovulation in the rat. Unpublished Ph.D. Thesis. University of Kansas. 1974.
9. Ho, G., D. M. Quadagno and H. Moltz. Intracranial cycloheximide: Effects on maternal behavior in the postparturient rat. *Pharmac. Biochem. Behav.* **2**: 455–458, 1974.
10. Lisk, R. D. Neural localization for androgen activation of copulatory behavior in the male rat. *Endocrinology* **80**: 754–761, 1967.
11. Lisk, R. D. Copulatory activity of the male rat following placement of preoptic-anterior hypothalamic lesions. *Expl Brain Res.* **5**: 306–313, 1968.
12. Lucas, L. A. and G. E. Abraham. Radioimmunoassy of testosterone in murine plasma. *Analyt. Letters* **5**: 773–778, 1972.
13. McGill, T. E. Genetic analysis of male sexual behavior. In: *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype*, edited by G. Lindzey and D. D. Thiessen. New York: Appleton-Century-Crofts, 1970, pp. 57–88.
14. McGill, T. E. and A. Manning. Genotype and retention of the ejaculatory reflex in castrated male mice. *Anim. Behav.*, in press.
15. Meyerson, B. J. Mechanisms of action of sex steroids on behavior: Inhibition of estrogen-activated behavior by ethamoxytriphetol (MER-25), colchicine, and cycloheximide. *Prog. Brain. Res.* **39**: 135–147, 1973.
16. Montemuno, D. G. and R. H. Dukelow. *A Stereotaxic Atlas of the Diencephalon of the Mouse.* New York: Futura Pub. Co., 1972.
17. Quadagno, D. M. and G. Ho. The reversible inhibition of steroid-induced sexual behavior by intracerebral cycloheximide. *Hormones Behav.* **6**: 19–26, 1975.
18. Sisler, H. D. and M. R. Siegel. Cycloheximide and other glutaramide antibiotics. In: *Antibiotics*, Vol. 1, edited by D. Gottlieb. Berlin: Springer, 1967, pp. 283–303.
19. Wallen, K., D. A. Goldfoot, W. D. Joslyn and C. A. Paris. Modification of behavioral estrus in the guinea pig following intracranial cycloheximide. *Physiol. Behav.* **8**: 221–223, 1972.