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Accumbens Lesion in Female Rats Increases Mount Rejection without Modifying Lordosis*

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Ovariectomized Wistar rats received bilateral electrolytic (n = 24) or sham (n = 11) lesion of the nucleus accumbens. Following priming with estradiol benzoate (25 µg/rat) and progesterone (0.5 mg/rat) they were tested for sexual behavior with a stud male. Tests were carried out once prior to operation and twice postoperatively. Both lordosis and rejection behaviors as responses to male mount attempts were evaluated for each session. Proceptive patterns (hopping, darting and presenting) were also recorded. Females with accumbens lesion did not differ from control animals either with regard to lordosis or to soliciting behaviors. On the contrary, the lesioned group showed a statistically significant increase in rejection behavior in both postoperative sessions ($p < 0.05$ and $p < 0.002$). In conclusion, nucleus accumbens lesion dissociated the normal correlation between lordosis and rejection responses to male mount attempts without affecting soliciting behaviors. This finding is thought to be related to the hyperreactivity produced by nucleus accumbens lesion.

Key words: Nucleus accumbens, Sexual behavior, Sexual receptivity, Rejection behavior.

Female rat sexual behavior results from the coordination of both reflexive and appetitive behaviors. The behavioral readiness of a female rat to accept and cooperate with males for copulating is manifested by receptive (lordosis reflex) and proceptive (hopping, darting and presenting) responses (7, 13). On the contrary, if a female rat is not sexually receptive, she will reject male mount attempts or will remain in a crouched posture during mounting (13, 15, 16).

The present experiments were performed to characterize the effects of nucleus accumbens lesion on female rat sexual behavior. The rationales for this study were: firstly, nucleus accumbens

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has been proposed as a component of neural mechanisms by which «motivation» is translated into «action», at the interface of the limbic system with motor mechanisms (9, 18), and female rat sexual behavior involves a complex coordination of both reflexive and appetitive behaviors (13); secondly, a role of the mesolimbic system in the regulation of both male copulatory behavior (4) and lordosis response (10) has been suggested, being nucleus accumbens a major target of mesolimbic axons and, thirdly, accumbens lesions produce marked hyperreactivity to a variety of stimuli in rats (2, 3, 12) and hyperreactivity has been involved in the apparent dissociation between two components of female sexual behavior in septal lesioned rats, lordosis and mount rejection (8).

Materials and Methods

Forty virgin female Wistar rats weighing 220-250 g at the beginning of the experiments were used. They were housed in pairs in a room kept at a constant temperature (22 ± 1 °C), and maintained on a 12 h light-dark cycle (lights on at 9.00 a.m.). Animals had free access to food and water.

Animals were ovariectomized a week after their arrival at the laboratory. They were brought into behavioral estrus by the sequential injection of 25 μg of estradiol benzoate and 0.5 mg of progesterone subcutaneously administered 48 h and 6 h respectively prior to testing.

Each single test started by placing individual subjects with a stud male in a methacrylate testing cage (37 × 29 × 25 cm). Females were removed when the 10th successful mount (mount with palpation and pelvic thrusts) was completed by the male. The lordosis strength in each of these mounts was rated on a scale from 0 to 3 (0, no lordosis; 1, marginal lordosis; 2, normal lordosis; and 3 for exaggerated lordosis). Lordosis quotient (LQ) was computed as the percentage of successful mounts in a test session for which a score of 2 or 3 was obtained (LQ = no. lordosis responses/10 × 100). To measure the female's mount avoidance, a rejection was scored if a female showed active resistance to a mount attempt resulting in the apparent abortion of the mount (8, 15). These rejection patterns included running away, rising on her hind legs, kicking off and any other aggressive patterns, from boxing to biting attack. A rejection quotient (RQ) was calculated by dividing the number of rejection responses by the total number of male mount attempts (successful mounts + unsuccessful mounts) and multiplying by 100 (15). The incidence of «hopping», «darting» and «presenting» which are considered to be proceptive, were also recorded. A proceptive index (PI) was computed for each session as the frequency of proceptive events per minute.

Tests were carried out once prior to operation and twice postoperatively during the last hour of the light phase. The preoperative test was performed on the 12th day after ovariectomy. Following this test, both LQ and RQ were computed for each female and those that did not show a LQ score > 70 and a RQ score < 20 were eliminated from the study. Postoperative observations were made ten days after de operation at a ten-day interval. Animal behavior was videotaped and analyzed by means of a software package made up to this end which calculated pat tern parameters, performed statistical analysis and ended up by printing out data and analysis results.

All surgery was performed under thiopenthal anesthesia (45 mg/kg b.w.). Electrolytic lesions were made by passing a 2 mA anodal direct current for 12 s through a 300 μm stainless steel electrode isolated with teflon except for 0.3 mm at the tip, and a silver-silver chloride cathode introduced through the caudal end of the surgical wound. Stereotaxic coordinates were

1.8 mm anterior to bregma, 7.5 mm below skull surface, and ± 1.3 mm from midline (19). Sham operations involved all surgical procedures except that the electrode tip was lowered to 1 mm above nucleus accumbens and no current was passed. When behavioral testing was completed, all animals were sacrificed and perfused intracardially with saline and formaline solutions. Frozen coronal sections 50 μm thick were mounted and stained with cresyl violet. The location and extent of the lesions were verified under microscope examination and transferred to the most appropriate diagrams of the stereotaxic atlas (19). Due to the heterogeneity of variance and the asymmetric distribution of data, they were subjected to the nonparametric Mann-Whitney U test. The Wilcoxon signed ranks test for correlated samples was used for comparison of preoperative PI with postoperative ones. For comparison of incidences of preceptive or aggressive rejection behaviors, the X^2 test with Yates' correction was used.

Results

Microscopic examination in sham operated animals showed gliosis along electrode tracts but did not show brain damage (control, n = 11). Lesions were found to be located entirely in the nucleus accumbens in twenty-four animals (lesioned, n = 24). Adjacent structures were also affected in five rats which were excluded from analysis. Figure 1 shows schematic diagrams indicating the limits within which all lesions were located and the extent of the damage for a representative case.

Both LQ and RQ results are shown in figure 2. Estrogen-progesterone treatment induced a high and similar level of receptivity (mean lordosis strength was always higher than 2) in both control and lesion groups. Intergroup differences were not statistically significant. On the contrary,

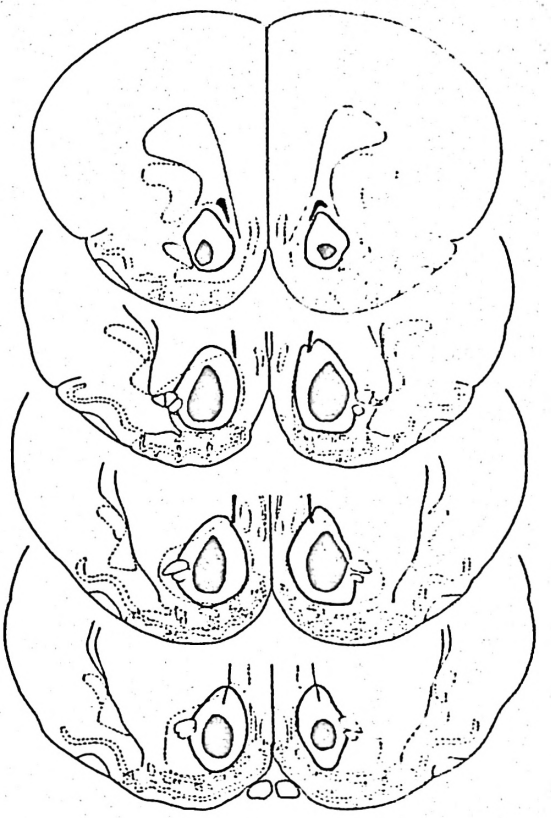

Fig. 1. *Schematic representation of the destroyed tissue.*
All lesions were included within the limits of the outer trace. The extent of the damage in rat R24 is shown in black. Stereotaxic diagrams from Paxinos and Watson (19).

although each one of the two experimental groups showed an increase in the rejection quotient in both postoperative sessions, the enhancement in the lesion group was higher than in the control group. These differences were statistically significant: postoperative 1, $U = 197$, $p < 0.05$; postoperative 2, $U = 209$, $p < 0.002$.

Lesioned females also exhibited qualitative differences in rejection behavior. Thus, aggressive rejection patterns were unusual in the control group (only 1/11 animals presented some aggressive rejec-

tion patterns in the first postoperative session and 2/11 in the second one) whereas they were very frequent in the lesioned females (12/24 and 10/24 animals showed such patterns for each postoperative session). However, intergroup differences in the incidence of aggressive rejections were statistically significant only for the first postoperative session ($X^2 = 4.188$, $p < 0.05$).

Data about proceptivity are presented in table I. Postoperatively, both groups showed a statistically significant decrease in the PI regarding preoperative levels (control: $T = 10$, $p < 0.05$ and $T = 6$, $p < 0.01$; lesion: $T = 55$, $p > 0.01$ and $T = 15$, $p < 0.001$, respectively for each postoperative session). However, intergroup differences were not significant either for the incidence of proceptive patterns or for the PI.

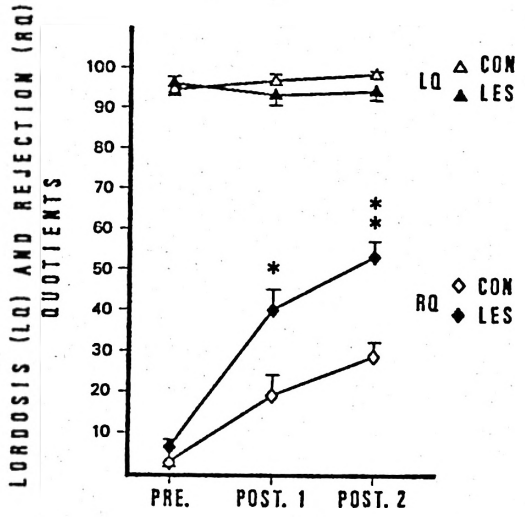

Fig. 2. *Lordosis quotient (no. of lordosis responses/no. of mounts with pelvic thrusting × 100) and rejection quotient (no. of rejection responses/no. of male mount attempts × 100), mean and s.e.m.* Ovariectomized rats were brought into estrus by the sequential administration of estradiol benzoate (25 μg) and progesterone (0.5 mg). Postoperative tests were separated by 10 days. Significant differences between groups: *$p < 0.05$; **$p < 0.002$ (Mann-Whitney U test).

Discussion

The present results show that nucleus accumbens lesion increased the number of rejection responses to male mount attempts without modifying the receptivity estimated by lordosis responses: lesioned females tried to avoid male mounts although they showed a strong lordosis response whenever the male managed to accomplish the mount.

Mating, under certain conditions, is known to have negative effects over later female rat copulatory behavior. These negative consequences have been related to the aversive effects of the female's impossibility of pacing the sexual interaction (11, 21) and to the penis intromission (6). In the present work, the increase in rejection showed by the controls throughout the experiments corroborate this idea.

Mesencephalic dopamine neurons that

Table I. *Proceptive behavior*

PI (mean ± s.e.m.) was computed for each session as the frequency of proceptive events per minute.

	Preoperative		Postoperative 1		Postoperative 2	
	Incidence	PI	Incidence	PI	Incidence	PI
Control	11/11	6.80 ± 2.64	10/11	3.95 ± 1.74*	10/11	1.88 ± 0.66**
Lesion	24/24	4.17 ± 1.47	21/24	4.08 ± 1.12**	16/24	1.08 ± 0.24***

Significantly lower than preoperative values (Wilcoxon signed ranks test): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

project to the nucleus accumbens are activated by arousing and complex environmental stimuli. This increased dopamine activity has been related to the feeling of anxiety or fear caused by the aversive nature of the stimulus (5). In this way, it has been suggested that the nucleus accumbens plays an adaptative role when the animal is faced with novel or aversive stimuli (14), and it is a key structure in the organization of the overall behavioral responses (9, 18).

In line with this notion, the nucleus accumbens lesion seems to diminish the female's tolerance to male mount and/or penis intromission. Thus, mount rejection in lesioned rats would be more related to the hyperreactivity to the stimulus (2, 3, 12), mount or intromission, than to sexual receptivity, although a direct consequence of this rejection behavior is the higher number of mount attempts to accomplish mating. Female rats with septal lesions exhibit high lordosis levels (8, 16, 17) and a simultaneous increase in mount rejection behavior and reactivity (1, 8). The parallelism of results following lesion is in accordance with the idea of rejection as a simple reflection of hyperreactivity, probably owing to the damage in their efferents to the hypothalamus (1).

It could be argued that reactiveness in lesioned animals should be declining over time with the handling they receive (3, 12). In fact, this decrease in reactiveness may explain that both groups were comparable in the incidence of aggressive rejection patterns in the second postoperative session. However, as normal reactive animals also increased the frequency of rejections, the aversive value of mount and/or intromission might be high enough to maintain such behavior even though the animals were comparatively less reactive in the second postoperative session than in the first one.

With regard to proceptive behavior, it is noteworthy that both groups showed a decrease in proceptive indexes after surgery. As proceptive patterns elicit sexual activity in males, an increase in rejection behavior should be followed by a decrease in the female's soliciting activity. Therefore, the aversive effects of previous copulatory activity would also explain these findings.

Nevertheless, a change in sexual motivation could be another alternative to explain the increase in rejection showed by lesioned animals. It is not inconceivable that accumbens lesion might have fractionated feminine sexual behavior by affecting sexual motivation and sparing lordosis and soliciting activity; other brain damage fractionate feminine sexual behavior by eliminating lordosis and soliciting behaviors and sparing the hormone dependent sexual motivation system (20).

In conclusion, it has been shown that nucleus accumbens lesion dissociates the normal correlation between lordosis and rejection responses to male mount attempts. Although this increase in rejection behavior may be the consequence of the hyperreactivity produced by nucleus accumbens lesion, an alteration in appetitive or motivational aspects of sexual behavior cannot be totally discarded from the present data.

Resumen

Se evalúan los efectos de la lesión electrolítica del núcleo accumbens sobre el comportamiento sexual de la rata hembra. Todos los animales se estudian una vez antes de la intervención y dos tras ella. Las hembras son ovariectomizadas y se utiliza la inyección secuencial de hormonas para la inducción del estro. En cada prueba se evalúa la respuesta de lordosis y los comportamientos de rechazo ante los intentos de monta del macho así como la frecuencia de pautas proceptivas. Mientras que ambos grupos son comparables en la respuesta lordótica y en el comportamiento proceptivo, los animales lesionados muestran una diferencia estadísticamente significativa en la inciden-

cia de rechazos en ambas sesiones postoperatorias ($p < 0.05$ y $p < 0.002$). Estos resultados se consideran relacionados con la hiperreactividad general producida tras la lesión del núcleo accumbens.

Palabras clave: Núcleo accumbens, Comportamiento sexual, Receptividad sexual, Comportamiento de rechazo.

References

1. Albert, D. J. and Richmond, S. E.: *Physiol. Behav.*, 15, 339-347, 1975.
2. Albert, D. J., Walsh, M. L., and Longley, W.: *Physiol. Behav.*, 35, 523-527, 1985.
3. Albert, D. J., Walsh, M. L., Zalys, C. and Dyson, E.: *Physiol. Behav.*, 38, 11-16, 1986.
4. Bitran, D. and Hull, E. M.: *Neurosci. Biobehav. Rev.*, 11, 365-389, 1987.
5. D'Angio, M., Serrano, A., Rivy, J. P. and Scatton, B.: *Brain Res.*, 409, 169-174, 1987.
6. De Bruijn, M., Broekman, M. and van der Schoot, P.: *Physiol. Behav.*, 43, 35-39, 1988.
7. Edwards, D. A. and Pfeifle, J. K.: *Physiol. Behav.*, 30, 437-443, 1983.
8. Gorzalka, B. B. and Gray, D. S.: *Physiol. Behav.*, 26, 39-44, 1981.
9. Heimer, L., Alheid, G. F. and Zaborszky, L.: In «The rat nervous system» (G. Paxinos, ed.), Academic Press, Sydney, 1985, Vol. 1, pp. 37-86.
10. Herndon, J. G., Jr.: *Physiol. Behav.*, 17, 143-148, 1976.
11. Krieger, M. S., Orr, D. and Perper, T.: *Behav. Biol.*, 18, 379-386, 1976.
12. Lee, S. C., Yamamoto, T. and Ueki, S.: *Behav. Neural Biol.*, 37, 237-245, 1983.
13. Lisk, R. D.: In «Handbook of Physiology», Sect. 7, vol. 2 (R.O. Green, ed.). American Physiological Society, Washington DC, 1973, pp. 223-260.
14. Louilot, A., Le Moal, M. and Simon, H.: *Brain Res.*, 397, 395-400, 1986.
15. Mathews, D. and Edwards, D. A.: *Physiol. Behav.*, 19, 319-326, 1977.
16. McGinnis, M. Y. and Gorski, R. A.: *Physiol. Behav.*, 24, 569-573, 1980.
17. McGinnis, M. Y., Nance, D. M. and Gorski, R. A.: *Physiol. Behav.*, 20, 435-440, 1978.
18. Mogenson, G. J., Jones, D. L. and Yim, C. Y.: *Prog. Neurobiol.*, 14, 69-97, 1980.
19. Paxinos, G. and Watson, C.: «The Rat Brain in Stereotaxic Coordinates», Academic Press, Sydney, 1982.
20. Pfeifle, J. K. and Edwards, D. A.: *Physiol. Behav.*, 31, 385-389, 1983.
21. Slob, A. K., de Klerk, L. W. L. and Brand, T.: *Physiol. Behav.*, 41, 571-576, 1987.