With regard to the high proportion of DBH the outer membrane structure of the protuberances resembles the inner side of the granular membrane. Thus, protuberances or microspikes might be interpreted as local enlargements of the cell surface, which occur especially after extensive stimulations leading to numerous inside-out integrations of granular membrane particles per an individual release spot. Normally the vesicle membrane may be quickly recycled. A continuous addition of membrane from integrated secretion organelles to the plasma membrane will lead to a localized enlargement of the cell surface (growth of protuberances), if the mechanism of membrane retrieval is interfered with. This seems to be the case with Ba2+ and BWSV but not, or to a lesser extent, with La<sup>3+</sup>. BWSV and barium are powerful stimulants of CA extrusion by acting directly on the chromaffin cell. Lanthanum in low concentrations facilitates CA release but blocks secretion at higher concentrations4.

In frog neuromuscular junctions treatment with BWSV was followed by a depletion of synaptic vesicles, which was explained by a blockade of vesicle recycling<sup>5</sup>.

Using Ba<sup>2+</sup> as a stimulant the formation of microspikes was more intensive when compared with K<sup>+</sup> given in high doses, although K<sup>+</sup> is a powerful stimulant of CA release. Also, in contrast to observations with potassium, they did not disappear after removal of the stimulus<sup>2</sup>.

- 1 J. Wildmann, M. Dewair and H. Matthaei, J. Neuroimmun. 1, 353 (1981).
- D.F. Englert, Exp. Cell Res. 125, 369 (1980).
- 3 P.U. Witte and H. Matthaei, in: Mikrochemische Methoden für neurobiologische Untersuchungen, p. 58. Springer, Berlin 1980.
- 4 J. L. Borowitz, Life Sci. 11, 959 (1972).
- 5 B. Ceccarelli, F. Grohovaz and W.P. Hurlblut, J. Cell Biol. 81, 163 (1979).

## Neonatal masculinization affects maternal behavior sensitivity in female rats<sup>1</sup>

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Summary. The present results indicate that maternal behavior in adult neonatally androgenized female rats is significantly diminished when compared to females at oestrus and intact males. In androgenized females cannibalism was detected.

It is a well established fact that the hormone environment during the early perinatal period determines whether a brain will function in a male or female fashion<sup>3</sup>. In this context a number of brain functions have been suggested to be the result of this process of sexual differentiation<sup>4</sup>. However, no attention has been payed to the possibility that neonatal masculinization may affect some behavior-patterns such as maternal behavior.

Female Wistar Holtzman rats were injected at day 5 of age with 1.25 mg testosterone propionate (androgenized rats) or oil (controls). Testosterone was administered s.c. dissolved in 0.1 ml of olive oil. Males from the same litter were used as normal males. 90 days after treatment the androgenized and control rats at oestrus were tested for maternal behavior after being caged together with 3 foster pups. The observation cages were made of glass and had a rectangular shape measuring 44 cm in length, 21 cm in width, and 20 cm in height. Animals were allowed 2-3 h of habituation to the observation cages before experiments were begun.

The following aspects of maternal behavior during a 2-h observation period were recorded:

- A) Licking: the animal must lick one or more pups for at least a total of 1 min.
- B) Grouping: the animal must carry in her mouth 3, 2-7-day-old pups and put them together in a corner.

Incidence of cannibalism in androgenized female rats

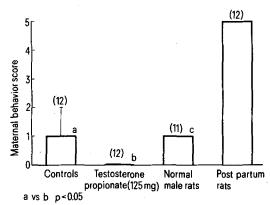
	No. of rats	No. of rats presenting cannibalism
Masculinized female rats Normal male rats	18 11	10 0

p ≤ 0.005.

C) Crouching: all pups must be grouped before crouching can be recorded. The animal must have its hind legs spread, its back arched, and its ventrum high enough off the cage floor to accommodate pups beneath her. There must be at least 1 pup beneath her during a crouch. This position must be displayed for at least a total of 1 min.

D) Nest building: the animal must pull more than half the 100 paper scraps into a corner where at least 1 pup has been or eventually will be carried (100 scraps were put in each cage during the habituation period).

E) Retrieval: any time within the 2-h observation period after grouping has taken place, 1 pup was separated by the observer to a distance of 15 cm from the female. The carrying of the pups back to the group within 15 min of separation constitutes retrieval.



Effect of neonatal androgenization on maternal behavior Data are expressed as the median of maternal scores. The vertical lines is the corresponding range. Numbers in parentheses indicate the number of animals per group.

Groups of rats were then assigned a behavioral score by giving each behavioral category 1 point in which the animal fulfilled minimal criteria. A score of 5 was considered fully maternal (postpartum rats).

Data were statistically analyzed by the Dunn and Mann Whitney U-test for nonparametric data and a probability of p < 0.05 was accepted as statistically significant.

As it can be seen in the figure, maternal behavior is significantly diminished in androgenized as compared to oil treated females. These masculinized rats showed a maternal score which was similar to normal male rats. Interestingly, neonatal androgenization caused a dramatic cannibalism,  $(p \le 0.005)$  which was not observed in either of the other groups (table).

These results show that the capacity to manifest maternal behavior towards foster pups can be affected by the process of sexual differentiation induced by androgens. These facts indicate that in the normal rat the maturation of the different mechanisms implicated in the control of maternal behavior take place during a critical period which extends between days 1 and 10 after birth.

The administration of a single dose of testosterone to female rats after birth produces an anovulatory syndrome with persistent oestrus<sup>5</sup>, lack of lordosis<sup>6</sup> and stimulation of male components of behavior<sup>7</sup>. From our results, it can be inferred that a true sexual differentiation of the brain has occurred, since the androgenized female behaved in a rather similar way to the normal male rat. These results are in agreement with some recent findings of Döhler and Hancke<sup>7</sup> which proposed that different amounts of oestradiol might bring about functional and structural changes of

the brain which would lead either to female or male differentiation

On the other hand, masculinization caused a cannibalistic behavior which was absent in normal male rats and in oil treated females. There is evidence that neonatal androgenization influences intraspecific aggression in rats<sup>8</sup> and in male and female mice<sup>9-11</sup>. However, further studies on the mechanism involved in the masculinization of the brain are necessary in order to understand the modification of the maternal behavior of androgenized female rats.

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- B. Flerkó, in: Growth and development of the brain, p. 117. Ed. M. A. B. Brazier. Raven Press, New York 1975
- R.A. Gorski, Frontiers in neuroendocrinology. Eds L. Martini and W.F. Ganong. Oxford University Press, New York 1971. R.A. Gorski, Biol. Reprod. 20, 111 (1979).

- F. A. Beach, Psychoneuroendocrinology 1, 3 (1975). K. D. Döhler and J. L. Hancke, in: Hormone and brain development, p. 153. Eds G. Dörner and M. Kawakami. Elsevier/ North Holland Biomedical Press, Amsterdam 1978.
- G.A. Barr, J.L. Gibbons and K.L. Moyer, J. comp. Physiol. Psychol. 90, 1169 (1976).
- F.S. vom Saal, B. Svare and R. Gandelman, Behav. Biol. 17, 391 (1976).
- R. Gandelman and F. vom Saal, Behav. Biol. 20, 252 (1977).
- R. Gandelman, Physiol. Behav. 15, 647 (1975).

## Calcemic responses of Stannius corpuscle extract in parrots Psittacula psittacula<sup>1</sup>

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Summary. In parrots (Psittacula psittacula), i.p. injection of Stannius corpuscle extract (10 mg/ml/100 g b.wt) evokes hypocalcemia at 1 h. Thereafter, the values indicate hypercalcemia at 6 h and normocalcemia at 10 h.

The corpuscles of Stannius (CS) are located on, or in, the kidneys of the holostean and teleostean fishes<sup>2</sup>. Stanniectomy in teleosts leads to hypercalcemia<sup>3-5</sup> which can be corrected either by homotransplantation of  $CS^{6,7}$  or by injections of corpuscular extracts<sup>3,7</sup>. It is now generally accepted that CS produce hypocalcemic principles called hypocalcin<sup>8</sup> and teleocalcin<sup>9</sup>.

Conflicting observations exist regarding the effects of CS extract administration in mammals. Both hypocalcemia 10 and hypercalcemia<sup>11</sup> have been reported after CS extract administration. Pang and Copp (both cited by Leung and Fenwick<sup>10</sup>) failed to observe any hypocalcemic action in rats after CS extract administration. So far, there is no record of the effects of CS extract on the serum calcium level of birds. The present work is a first report on such a study in the parrot, Psittacula psittacula.

Material and methods. The CS used in this study were surgically removed from both sexes of an adult freshwater mud eel, Amphipnous cuchia. These glands were stored in ice and used almost immediately. The glands were weighed and homogenized in ice-cold saline (0.9% sodium chloride solution). The homogenate was centrifuged (5000 rev/min for 10 min) and the supernatant was collected. The final volume of the supernatant was made up so that 1 ml of the

solution contained the extract from 10 mg of wet CS. 72 parrots (Psittacula psittacula), weighing from 90 to 110 g, were maintained on paddy under laboratory conditions for 2 weeks prior to use. They were then divided into 2 numerically equal groups a) saline-injected (control); and b) CS extract-injected (experimental).

The experimental specimens were injected i.p. with CS extract in a dosage of 10 mg/ml/100 g b.wt. The control specimens were injected i.p. with 1 ml/100 g b.wt of saline. Blood samples from both the groups were collected by cardiac puncture after 0.5, 1, 2, 4, 6 and 10 h following the injection. The concentration of calcium was measured in the serum by Trinder's 12 method. Calcium from a 0.2 ml sample of serum was precipitated as an insoluble orangered complex by an alkaline solution of naphthalhydroxamic acid. After centrifugation the precipitate was dissolved in alkaline disodium ethylenediamine tetraacetate, then treated with ferric nitrate and the resultant amber color was measured colorimetrically. Calcium concentration has been expressed as mg/100 ml serum.

Differences between parrots injected with saline and with CS extract were evaluated using Student's t-test.

Results and discussion. The serum calcium level is unaffected at 0.5 h after the injection of CS extract. At 1 h following