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Electrolytic lesions to the anterior hypothalamus-preoptic area disrupt maternal nest-building in intact and ovariectomized, steroid-treated rabbits

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1. Introduction

Maternal behavior in rabbits begins with the construction of a maternal nest where the doe will deliver the litter and nurse it throughout lactation (González-Mariscal et al., 1994, 2016). In nature, in the laboratory, and on the farm nest-building begins in mid-pregnancy as the doe digs an underground burrow in the field or into a substrate placed within the nest-box in the female's home cage. This activity is followed by the collection of straw or hay - taken from the field or a container- with which the mother builds the so-called straw nest within the burrow or nest box. Hair-plucked mainly from the ventrum and inner thighs - is then used as a lining to complete the maternal nest (González-Mariscal et al., 2007, 2016). The onset and offset of digging, straw-carrying, and hair-plucking is tightly controlled by the changing concentrations of hormones in blood across pregnancy, specifically: estradiol, progesterone, prolactin, and testosterone (González-Mariscal et al., 1994). Moreover, in ovariectomized (ovx) does the combined injection of estradiol benzoate (EB) plus progesterone (P) stimulates digging while P removal provokes a decline in digging and the onset of straw-carrying, followed by hair-plucking (González-Mariscal et al., 1996). This sequence of activities replicates the behavior of pregnant does from mid-gestation to parturition and supports a major role of estradiol and P in timing the expression of maternal nest-building.

Receptors for such gonadal steroids are present throughout the female rabbit forebrain, including the preoptic region, anterior hypothalamus, and bed nucleus of the stria terminalis (BNST) (Caba et al., 2003 a, b). In ovx does, bilateral implants of EB into either the preoptic region or BNST stimulate digging - when accompanied by subcutaneous (s.c.) P injections- while interruption of P treatment leads to straw-carrying and a decline in digging. Yet, P implants into either of those regions fail to stimulate digging in ovx rabbits given EB s.c. (González-Mariscal et al., 2005). These results indicate that the action of estradiol on the preoptic region-BNST is sufficient for P-facilitation of digging and straw-carrying. However, the question remains regarding the relevance of those brain regions for the action of P on estradiol-dependent nest-building. Thus, in the present work we explored if electrolytic lesions to the anterior hypothalamus-preoptic (AH-PO) region would antagonize the capacity of P injections to stimulate nest-building in two types of doe rabbits: a) intact, exposed to their own ovarian estrogens; b) ovx injected s.c. with EB. We also investigated, in ovx EB-primed does, whether such lesions interfered with the expression of lordosis and scent-marking, as these behaviors are stimulated by EB only (and inhibited by P). Therefore, differential effects of AH-PO lesions on nest-building vs estrous behavior would indicate differences in the neural substrate underlying P-stimulated vs estrogen-stimulated behaviors, respectively.

2. Material and methods

2.1. Animals

Adult New Zealand white nulliparous rabbits (3.0–3.5 Kg body weight) bred in our colony were used. They were housed in maternal wire mesh cages (90 cm long × 60 cm wide × 40 cm high) inside the rabbit colony under controlled light (14 L:10 D; lights off at 2100 h) and natural temperature conditions. Females were given 300 g of Purina™ rabbit pellets and 1000 ml of water/day. Throughout this experiment animal care adhered to the Law for the Protection of Animals (Mexico) and with international guidelines regarding animal research (González-Mariscal et al., 2017).

2.2. Surgeries

Rabbits were ovx under ketamine (25 mg/Kg)/rompun (8 mg/Kg) anesthesia using sterile conditions and, after the operation, the received 200,000 units of penicillin i.m. For stereotaxic surgeries, to lesion the anterior hypothalamus-preoptic (AH-PO) region in both intact and ovx does, animals were anesthetized as above and an insulated stainless steel electrode (0.405 mm diameter, gauge 26; 5.0 cm long) was lowered into the brain using the following coordinates from the atlas of Girgis and Shih-Chang (1981): anterior = 2.0; lateral = ± 1.0; dorso-ventral = 13.5. Electrolytic lesions were made by applying direct current (square pulses of 3.0 mAmp for 30 s) on each right and left sides.

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2.3. Drugs

EB and P were purchased from Sigma (St. Louis, MO, USA). Both steroids were dissolved in sesame oil to a concentration of 10 μg/ml (EB) or 20 mg/ml (P) for s.c. injections.

2.4. Behavioral measurements

2.4.1. Nest-building

A wooden nest-box (50 cm long × 30 cm wide × 32 cm high) with a round (24 cm diameter) opening on one side was introduced into the female's home cage to quantify the three activities involved in constructing the maternal nest, as earlier described (González-Mariscal et al., 1994): a) digging: a piece of compressed cardboard (cut to cover the entire floor of the nest box) was weighed, introduced into the wooden box, left inside it for 24 h, removed, and weighed again. The decrease in the weight of the cardboard indicated the amount of substrate dug in a day. b) straw-carrying: 100 g of straw were placed inside the female's cage (but out of the nest box) and, 24 h later, the straw introduced into the nest box was removed and weighed. c) hair-plucking: the nest box was inspected each day to determine the presence of hair tufts. If present, they were removed and weighed.

2.4.2. Body weight and temperature; food and water intake

As electrolytic lesions to the diencephalon (including the AH-PO region) of female rabbits have been reported to induce hyperthermia, loss of appetite, decrease in body weight, and even death (Sawyer, 1959), we assessed the does' health state before and after lesions by determining, on specific days: their body temperature and weight as well as their intake of food and water.

At 10:00 h does received the daily portion of food and water (see above) and, 24 h later, the amount of pellets and water remaining in the food container and bottle, respectively, were measured. This allowed us to determine the daily intake of food and water. Body temperature was measured with a rectal thermometer and body weight by placing the animals on a manual scale.

2.5. Experiment 1: effect of s.c. P injections to intact rabbits before and after electrolytic lesions

As rabbits are reflex ovulators they are continuously exposed to estrogens, produced by the maturing follicles. Following mating, ovulation occurs, a corpus luteum is formed and P secretion begins (Ramírez and Beyer, 1988). This condition, therefore, offers the advantage of allowing us to compare in *unmated* does their responsiveness to *only* exogenous P (i.e., without the additional injection of estradiol) before vs after lesions. This experimental model more closely resembles what occurs in estrous rabbits following mating as they transition from estrus into pregnancy (Hoffman and González-Mariscal, 2007). To induce nest-building *before* lesions intact unmated rabbits (n = 8) were injected daily with 10 mg P from days 1 to 14. This dose of P was selected based on our earlier work showing its effectiveness for inducing nest-building in ovx EB-primed rabbits (González-Mariscal et al., 1996). Digging, straw-carrying, and hair-plucking were quantified as described above from days 1 to 22. The rabbits' body weight and temperature were determined on days 1, 7, and 14. Food and water intake were quantified daily. At the end of observations (day 22) does were left undisturbed for 7 days to "wash off" the administered P. As the last P injection occurred on day 14 the total number of days without this hormone was 15. At this point does were bilaterally lesioned as described above and, on the following day, they started receiving the same P treatment as earlier (i.e., 10 mg P/day for 14 days). Nest-building and the same physiological and somatic parameters were again measured as before.

2.6. Experiment 2: effect of s.c. P injections to ovx EB-treated rabbits before and after lesions

As s.c. injections of EB and P effectively induce maternal nest building in ovx does (González-Mariscal et al., 1996) we used the same hormonal regime to compare the rabbits' responses before vs after lesions. Three weeks after ovariectomy does (n = 17) were daily injected s.c. with 5 μg EB/day for 26 days. P (10 mg/day) was administered s.c. from days 6 to 19. The same behaviors, physiological, and somatic parameters quantified in Experiment 1 were assessed across days 1 to 26. In addition, ambulation in an open field and two components of estrous behavior, i.e., scent-marking (chinning) and sexual receptivity (lordosis), were quantified on days 5, 7, 14, and 26, as estrous behaviors are stimulated by estradiol and inhibited by P in both intact (González-Mariscal et al., 1990) and ovx rabbits (Hudson et al., 1990). Chinning frequency and ambulation were quantified simultaneously inside a square wire mesh arena (1 m × 1 m) with a grid-painted floor that contained three piles of bricks, as described earlier (García-Dalmán and González-Mariscal, 2012). Across 10 min we determined: a) the number of times the female rubbed its chin onto any of the three brick piles and b) the number of grid lines crossed. At the end of this test, females were transferred to a round wire mesh arena (1 m in diameter) to quantify sexual receptivity. The lordosis quotient was determined by counting the number of times a female showed the lordosis posture in response to three mounts made by a sexually active male. At the end of observations (day 22) does were left undisturbed for 7 days to "wash off" the administered P. Does were then bilaterally lesioned as described above and, one week later, they received again 5 μg EB/day for 26 days plus P (10 mg/day) from days 6 to 19. The rabbits' body weight and temperature, their food and water intake were quantified on the three days before and three days after the lesions. Nest-building and the same physiological and somatic parameters were measured as before.

2.6.1. Determination of "global" vs activity-specific effects of lesions

As nest-building involves the sequential expression of three activities (digging, straw-carrying, hair-plucking) intracerebral lesions could have – theoretically-altered one, two or three of them. Moreover, lesions could have provoked a decrease, an increase, or no change in those activities. Therefore, in order to more accurately describe the effects provoked by brain lesions and to associate them with a specific location in each individual, we devised a "disruption score" (DS) ranging from 0 to 3 indicating the number of nest-building activities that were reduced post-lesion by at least 25%, relative to pre-lesion values. Reciprocally, a "facilitation score" (FS; also ranging from 0 to 3) indicated the number of nest-building activities that were facilitated post-lesion by at least 25%, relative to pre-lesion values. We then calculated a 'General Effects" index from the quotient DS/FS + 1.

2.6.2. Perfusion and tissue preparation

To determine the placement and size of electrolytic lesions at the end of each experiment does were anesthetized with sodium pentobarbital (approximately 60 mg/Kg) and perfused transcardially with 0.9% saline followed by 20% formaldehyde. Brains were then removed, cryoprotected successively in 10%, 20%, and 30% sucrose, and sectioned coronally at 40 μm in the coronal plane with a cryostat from the rostral border of the preoptic area to the mammillary bodies. One of every six sections was stained with cresyl violet for visualization of neuroanatomical structures. Camera lucida drawings were made of all sections and the location of lesions was indicated therein.

2.6.3. Statistical analysis

Pre- and post-lesion measures for all behaviors (food and water intake, chinning frequency, lordosis quotient, digging, straw-carrying, and hair plucking) were compared using the Wilcoxon signed-rank test (SPSS Software, v.15.0 for Windows). In order to assess lesion effects on P responsiveness (i.e., responsiveness to the administration of P, as well