

DECREASED ALLOPREGNANOLONE INDUCED BY HORMONAL CONTRACEPTIVES IS ASSOCIATED WITH A REDUCTION IN SOCIAL BEHAVIOR AND SEXUAL MOTIVATION IN FEMALE RAT

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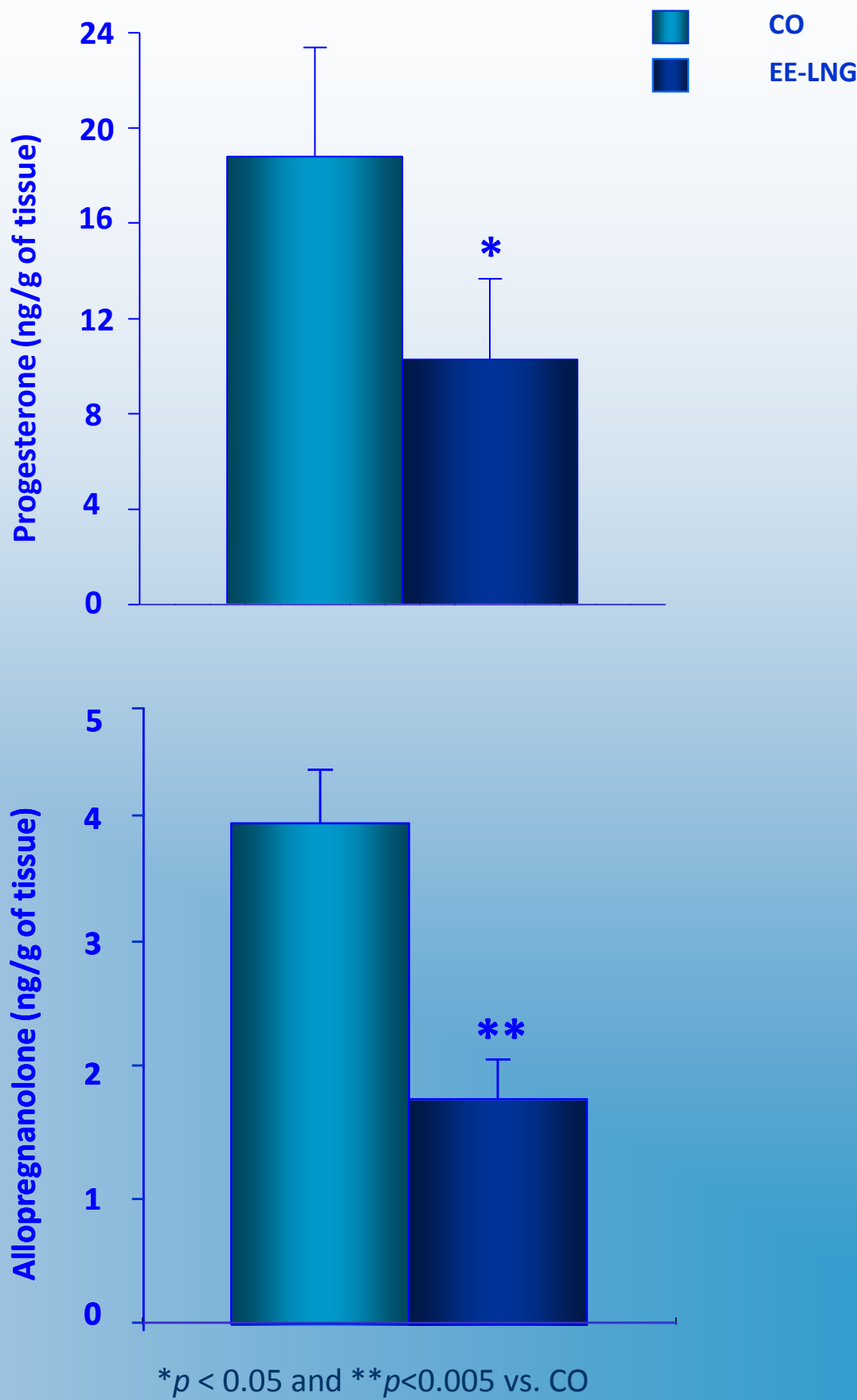
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INTRODUCTION

Allopregnanolone is a neuroactive steroid, produced in the periphery and directly in the brain from progesterone, which exerts a rapid non genomic effect on neuronal excitability through a direct modulation of the type A receptor of gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain¹. It has been shown that this steroid facilitates social and sexual behaviour of rodents, which evoke further increases in brain allopregnanolone concentrations². Thus, systemic or local allopregnanolone administration in the hypothalamus or midbrain, promotes sexual behavior, while blocking progesterone metabolism through a 5 α -reductase inhibitor, reduces proceptivity and receptivity compared to control animals^{2,3}. We have previously demonstrated that chronic treatment with a combination of ethinyl estradiol (EE) and levonorgestrel (LNG), two of the compounds most frequently used in the preparation of oral contraceptives (OCs) , once daily for 4 weeks to female rats, induces a decrease in brain and plasma concentrations of allopregnanolone and progesterone⁴. We now examined whether the reduction in the concentrations of allopregnanolone induced by the EE-LNG treatment was associated with a modification in the social and sexual behavior of rats.



*p < 0.05 and **p < 0.005 vs. CO

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MATERIALS AND METHODS

Animals and pharmacological treatments

Female rats were treated orally with a combination of ethinylestradiol (EE, 0.030 mg/ml carboxymethylcellulose 0.4%) and levonorgestrel (LNG, 0.125 mg/ml carboxymethylcellulose 0.4%) once a day for 4 weeks. Control rats received the same volume of vehicle. Animals were maintained on reversed 12-h-light, 12-h-dark cycle (light on from 20.00 to 8.00 hours) and sacrificed or subjected to the behavioral tests 24 hours after the last treatment. The female rats used as intruders in the resident-intruder test were ovariectomized during equithesin anaesthesia (pentobarbital, ethanol 96%, and chloral hydrate; 3.5 ml/Kg) two weeks before test.

Resident intruder paradigm

This test is used to study the display of offensive aggressive behaviors of an animal (resident) against an unfamiliar conspecific (intruder) which is introduced in its territory. Experimental animals (residents) were housed individually and no cage changes took place until testing, allow the residents to establish a territory in their home cages. The day of the test, an ovariectomized female intruder was introduced into the resident's home cage and animals were videotaped for 15 min for subsequent behavioral analysis of the social interactions based on the ethogram by Grant and Mackintosh⁵, using the Behavior Tracker software.

Non Paced-mating

Each experimental animal was placed with a "vigorous" male in a single cage a whole night, for 4 consecutive nights and the interaction was videotaped for the subsequent analysis of female sexual behavior. Female receptivity, expressed as lordosis quotient (LQ, lordosis / mounts + intromissions X 100), and proceptivity, were measured.

Paced Mating

Mating occurred in a chamber divided in two half by a partition with an escape-re-entry hole through which only females, by virtue of their size, could pass and avoid males. In the first experiment paced mating behavior was assessed in EE-LNG- treated female rats and in control female rats in randomized phases of estrous cycle; in the second experiment, paced-mating behavior was assessed in EE-LNG- treated female rats treated with: vehicle (200 μ l sesame oil s.c.), β -estradiol 3-benzoate (EB, 10 μ g/rat/200 μ l sesame oil s.c.) 48 hours before test, progesterone (P; 4 mg/Kg/200 μ l sesame oil s.c.) 4 hours before test, finasteride (F; 50 mg/kg/3 ml sesame oil s.c.) 6 hours before test. Animals were videotaped for 20 minutes and receptivity and proceptivity were then analyzed.

Steroids extraction and measurement

EE-LNG-treated rats were sacrificed after SC treatment with: vehicle (200 μ l sesame oil), β -estradiol 3-benzoate (EB; 10 μ g/rat/200 μ l sesame oil) 48 hours before sacrifice, progesterone (P; 4 mg/Kg/200 μ l sesame oil) four hours before sacrifice, finasteride (F; 50 mg/kg/3 ml sesame oil) six hours before sacrifice followed two hours later by P and F alone. Steroids were extracted and purified as previously described⁶. Steroids present in the cerebrocortical homogenate, were extracted four times with ethyl-acetate (1:1.5, vol/vol). The combined organic phases were dried under vacuum, the resulting residue was dissolved in 4ml of n-hexane and applied to Seppak-silica cartridges (Waters) and residual components were eluted with a mixture of n-hexane and 1-propanol (7:3, vol/vol). Steroids were further purified by high-performance liquid chromatography (HPLC) on a 5- μ m Lichrosorb-diol column (250 by 4mm) (Phenomenex) with a gradient of 1-propanol in n-hexane. The recovery of each steroid through the extraction-purification procedures (70 to 80%), was monitored by adding trace amounts (6000 to 8000 cpm) of ³H-labeled standards to the brain tissue homogenate. Steroids were quantified by radio immunological assay (RIA) as previously described⁶.

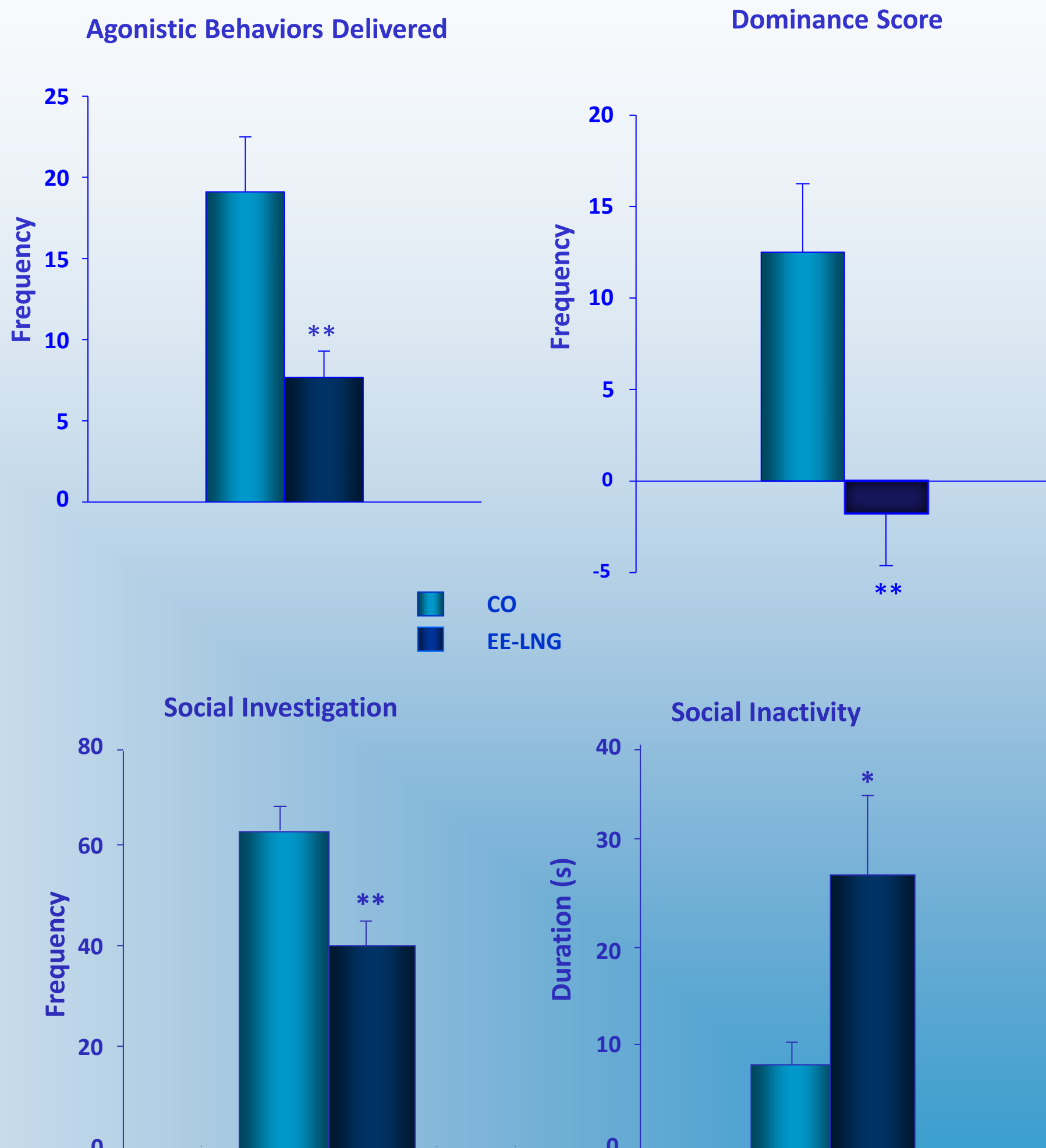
Statistical analysis

Data are means \pm SEM. The statistical significance of differences was assessed by one-way or two-way analysis of variance (ANOVA) and a post-hoc analysis was performed by Newman-Keuls test. A value of p<0.05 was considered statistically significant.

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EFFECT OF LONG-TERM TREATMENT WITH EE-LNG ON THE BEHAVIOR OF RATS IN THE RESIDENT-INTRUDER TEST

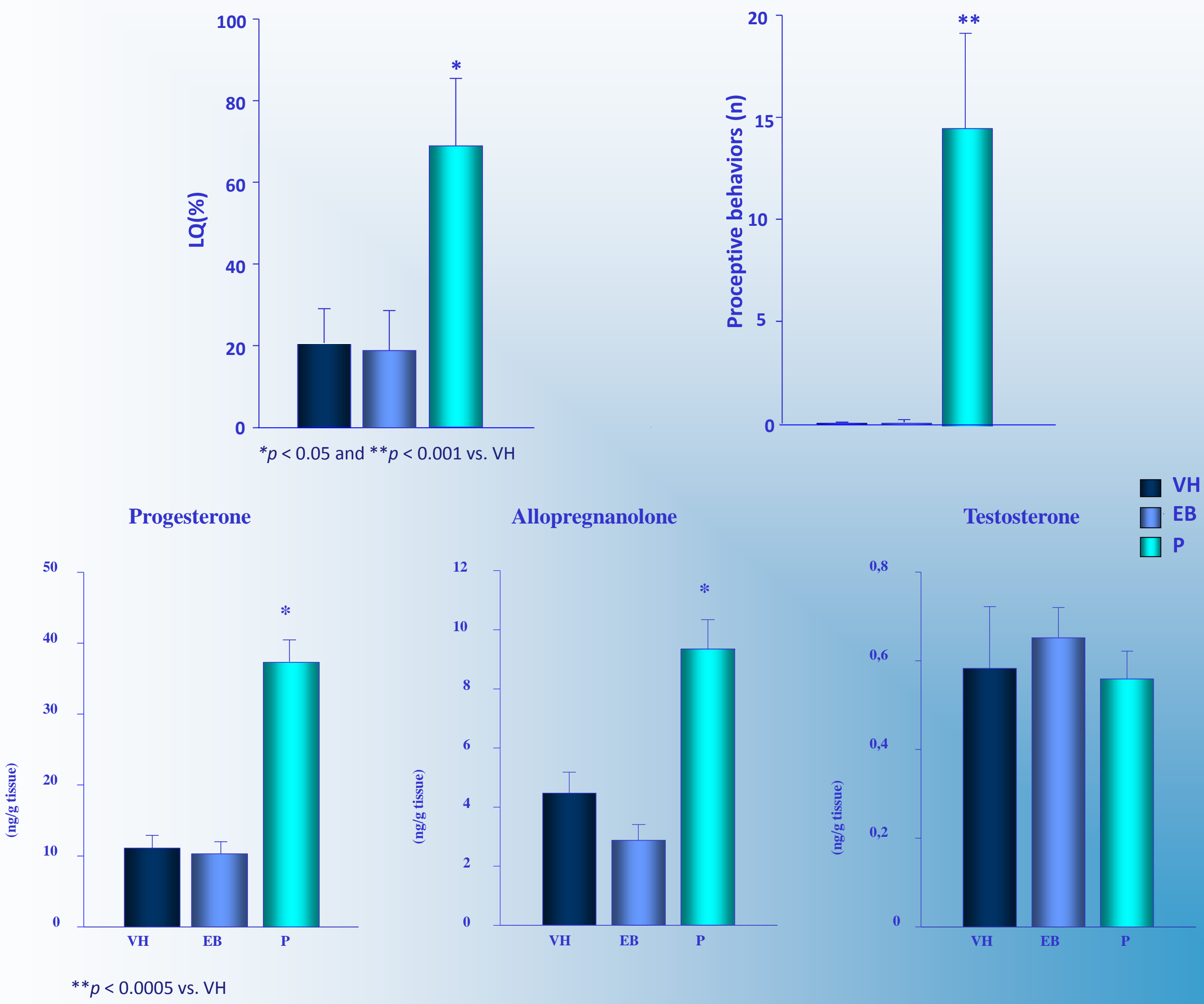
Scored behavior	Control	EE-LNG
Chasing the intruder		
Frequency	8.5 \pm 1.37	4.3 \pm 0.87*
Dominant behaviors		
Frequency	10.5 \pm 2.16	3.44 \pm 1.06*
Attacks delivered		
Frequency	0.1 \pm 0.1	0.4 \pm 0.31
Submissive behaviors		
Frequency	2.22 \pm 0.64	1.9 \pm 0.43
Body investigation		
Frequency	38.7 \pm 2.25	24.9 \pm 2.68**
Anogenital investigation		
Frequency	10 \pm 1.35	2.8 \pm 0.93**
Total social behaviors		
Frequency	28.7 \pm 4.44	21.1 \pm 2.51
Total agonistic behaviors		
Duration (s)	133.8 \pm 30.65	58.4 \pm 13.13*
Non-social behaviors		
Duration (s)	536.3 \pm 46.67	671 \pm 33.54*



*p < 0.05 and **p < 0.01 vs. Control

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EFFECT OF ESTRADIOL AND PROGESTERONE ADMINISTRATION ON THE BEHAVIOR OF EE-LNG-TREATED RATS IN THE PACED MATING TEST



**p < 0.0005 vs. VH

4

EFFECT OF LONG-TERM TREATMENT WITH EE-LNG ON THE BEHAVIOR OF RATS IN THE NON-PACED MATING TEST



Each experimental animal was placed with a "vigorous" male in a single cage a whole night, for 4 consecutive nights and videotaped for the subsequent analysis of female sexual behavior. Female receptivity, expressed as lordosis quotient (LQ, lordosis / mounts + intromissions X 100), and proceptivity, were measured.

*p < 0.05 vs. Control

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EFFECT OF LONG-TERM TREATMENT WITH EE-LNG ON THE BEHAVIOR OF RATS IN THE PACED MATING TEST

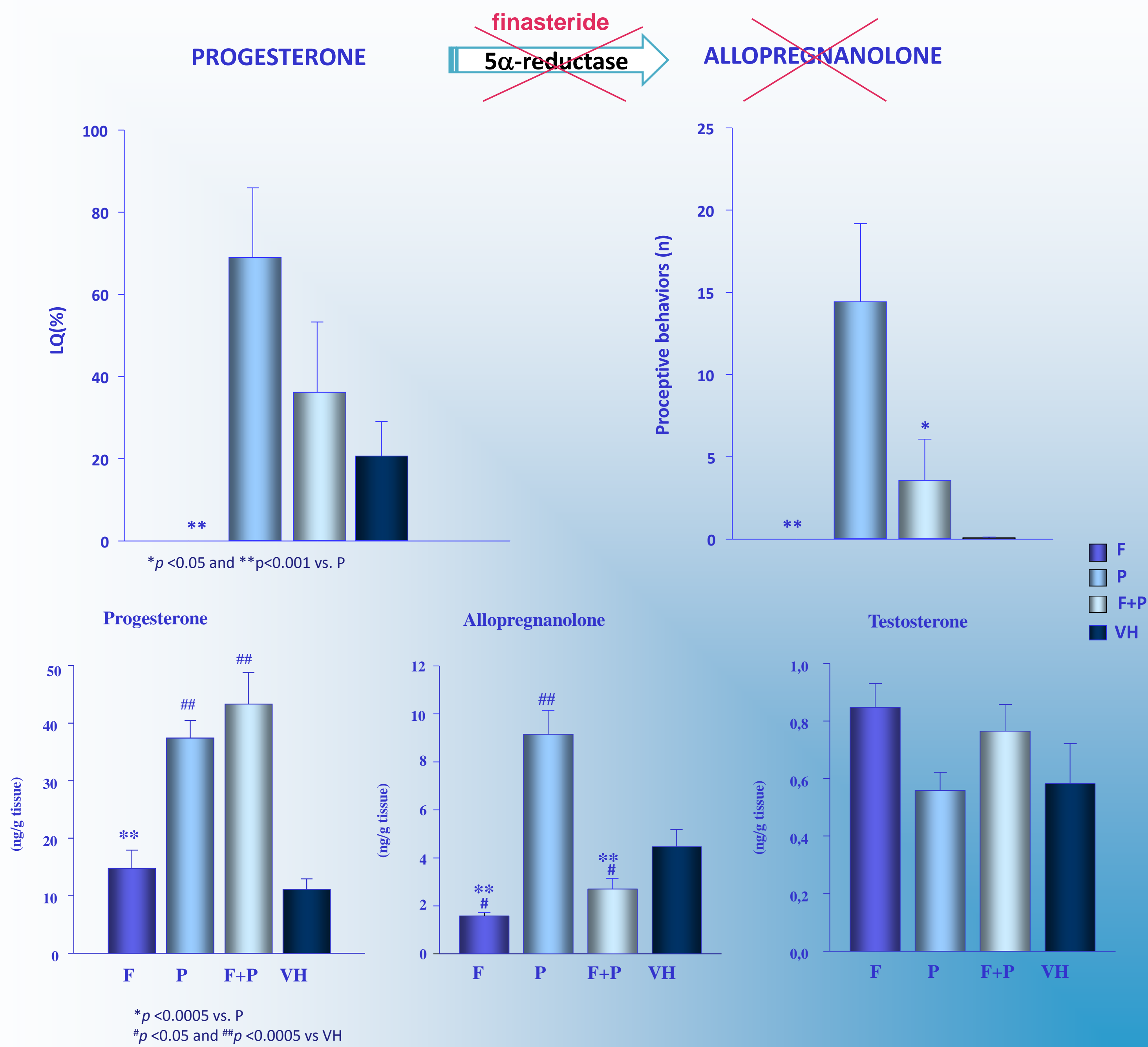


Mating occurred in a chamber divided in two half by a partition with an escape-re-entry hole through which only females, by virtue of their size, could pass and avoid males. Control female rats were in randomized phases of estrous cycle. Female receptivity, expressed as lordosis quotient (LQ, lordosis / mounts + intromissions X 100), and proceptivity, were measured.

*p < 0.05 vs. Control

7

EFFECT OF FINASTERIDE ADMINISTRATION ON THE BEHAVIOR OF EE-LNG-TREATED RATS IN THE PACED MATING TEST



*p < 0.0005 vs. P
#p < 0.05 and ##p < 0.0005 vs. VH

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CONCLUSIONS

- ✓ EE-LNG-treated rats showed significant decreases in dominant and social behaviors in the resident-intruder paradigm. Given that these behaviors are dependent upon the hormonal fluctuations during the estrous cycle, our results may be related to the lack of these fluctuations induced by EE-LNG.
- ✓ Long-term treatment with EE-LNG specifically affected sexual motivation in both Non-paced mating and Paced-mating tests, as shown by the drastic reduction in proceptive behaviors. This is related to the low allopregnanolone concentrations measured in the brain of treated animals, which exerts an important role in sexual motivation.
- ✓ Estradiol administration to EE-LNG-treated females did not affect the behavior of rats in the paced-mating test or the cerebrocortical allopregnanolone concentrations, while progesterone treatment resulted in significant increases in both receptivity and proceptivity. This could be related with the recovery of brain concentrations of allopregnanolone induced by progesterone administration.
- ✓ The administration of finasteride, alone or in combination with progesterone, resulted in a significant decrease in proceptive behaviors with respect to P-treated rats, and in a drastic reduction of allopregnanolone concentrations, while it did not alter progesterone levels. This results suggest that allopregnanolone is necessary for the expression of proceptive behaviors, and its low levels are responsible for the reduction of sexual motivation in EE-LNG-treated rats.
- ✓ These effects might be relevant to the side effects sometimes exhibited by women taking hormonal contraceptives.

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