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The transition to reproductive senescence is characterized by increase in A6 and AVPV neuron activity with attenuation of noradrenaline content

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ABSTRACT

During the course of life, cyclic females face a state of midlife transition that occurs in a fully functioning neurological system, and results in reproductive senescence. The authors' hypothesis was that changes in the activity noradrenergic neurons may be one of the factors involved in this phenomenon. The aim of this study was to investigate the activity of the neurons in the anteroventral periventricular nucleus (AVPV) and locus coeruleus (LC), to analyze their role in determining reproductive senescence. Adult female Wistar rats in the diestrus phase (4 months/cyclic) and old females (18–20 months/acyclic) in persistent diestrus, were decapitated or perfused at three different time intervals (10, 14 and 18 h) throughout the day. In acyclic rats, the gonadotropin-releasing hormone (GnRH) and noradrenaline (NE) content were reduced; *Fos*-related antigen (FRA) in AVPV and *Fos*-related antigen/Tyrosine hydroxylase (FRA/TH) in LC showed immunolabeling of a higher number of neurons in these animals. The 3-methoxy-4-hydroxyphenylglycol/noradrenaline (MHPG/NE) ratio was higher and plasma LH was lower in the acyclic rats. Furthermore, the estradiol level was higher, and the progesterone level was lower after 14 h of persistent diestrus. These findings suggested that during the periestropause, there was a higher level of POA/AVPV and NE neuronal activity in the LC of acyclic rats, associated with a lower capacity of synthesis and storage of neurotransmitters and neurohormones contributed to changes in the temporal pattern of neuroendocrine signaling, thereby compromising the accuracy of inhibitory and stimulatory effects, causing irregularity in the estrous cycle and determining reproductive senescence

1. Introduction

The state of transition in cyclic females, such as perimenopause, is characterized by an integrated series of phase-dependent transformations that involve sequential activation and deactivation of complex regulatory pathways (Petricka and Benfey, 2011). With advanced female aging, there are further reproductive changes, typically a loss of reproductive capacity with partial or complete reproductive senescence. During this process, each level of the hypothalamic-pituitary-gonadal (HPG) axis undergoes changes in structure, function, and hormone synthesis/release (Wang et al., 2015). All women who reach the age of 60 years with their reproductive organs intact will go through perimenopause to menopause with duration of 1–5 years from start to completion (Brinton, 2010; Harlow et al., 2012). These states of transition are associated with regulatory network restructuring that leads to reproductive senescence, and in the females of most species, this results from changes in the HPG axis (Brann and Mahesh, 2005; Downs and Wise, 2009; Neal-Perry et al., 2010).

Rodent and nonhuman primates, and humans share the common features of perimenopausal transition, with irregular cycling and fertility, steroid hormone fluctuations and lower sensitivity to estrogen (Diaz Brinton, 2012; Finch, 2014). Delayed onset and attenuated amplitude of the preovulatory LH surge, in rodents, occurs from 8–12-months of age and extends up to 18 months, when the persistent diestrus phase begins, after a brief period of constant estrus (Ferreira et al., 2015), and characterizes onset of reproductive senescence (Acuña et al., 2009; Cora et al., 2015). However, acyclic rodents are able to return ovarian function in response to stimuli in gonadotropin releasing hormone (GnRH) neurons (Campos and Herbison, 2014), suggesting that neuroendocrine components contribute to determining the reproductive aging (Cashion et al., 2003; Franceschini and Desroziers, 2013; MohanKumar and MohanKumar, 2004). Attenuated GnRH neurosecretion from the medial basal hypothalamus (MBH) (Rubin, 2000), and altered secretion of neurotransmitters such as glutamate (Neal-Perry et al., 2005); GABA (Mitsushima et al., 2002); noradrenaline (NE) (MacKinnon et al., 1983); and kisspeptin (Dungan et al., 2007;

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Gianetti and Seminara, 2008), may be associated with the female states of transition (Le et al., 2001).

The important action of the NE in controlling the preovulatory LH surge of reproductive-aged rodents occurs via the preoptic area (POA), principally the anteroventral periventricular (AVPV) nucleus, that receives projections of noradrenergic neurons from the locus coeruleus (LC), *A*1 and A2 (Campbell and Herbison, 2007; Szawka et al., 2009; Williams and Kriegsfeld, 2012). Electrolytic lesions in the LC decrease NE content in the medial POA and MBH, disrupt the estrous cycle and block the preovulatory surge of gonadotropin (Anselmo-Franci et al., 1997). Moreover, several studies have shown the participation of NE in the GnRH/LH surge during the reproductive period. Nevertheless, this has not been elucidated during the state of transition from the cyclic to acyclic stage, characterized by the non-occurrence of the LH surge. The authors hypothesized that in the aging female, changes in the activity noradrenergic neurons may be one of factors involved in determining the reproductive senescence.

Therefore, the authors analyzed and compared the activity of AVPV neurons and noradrenergic neurons of the LC; concentrations of the NE and 3-methoxy-4-hydroxyphenylglycol (MHPG)/NE ratio, in addition to the GnRH content in the MBH of cyclic adult rats, in diestrus, and in persistent diestrus of acyclic old rats, in order to elucidate their participation in reproductive aging. The authors performed in vivo experiments using rats in natural aging to analyze the real changes that occurred in this period and determined reproductive senescence.

2. Methods

2.1. Animals

Ninety-six female rats of the Wistar strain, aged 4 months (adult-cyclic) and 18–20 months (old-acyclic), were housed in plastic cages, in groups of four, in a temperature-controlled room (22 ± 2 °C) with a 12/12 h light/dark cycle (lights on at 7:00 h). Food and water were provided ad libitum. Vaginal smears were taken daily for analysis of the estrous cycle. Animal experiments were carried out in accordance with the laboratory principles of animal care (Council, 2011) and were approved by the local Ethics Committee for Research Involving Animals of the University Estadual Paulista (CEUA: 01829-2011). Only adult rats (4 months) showing normal estrous cycles, and old rats (18–20 months) in persistent diestrus, showing at least three consecutive cycles, were used in this study. The criteria for inclusion in this study were: the use of only multiparous female rats and those in the diestrus phase. All female rats were sacrificed at 10, 14 and 18 h on the diestrus day, in order to estimate neuronal activity of both the AVPV and LC, by using FOS protein expression as a marker of neuronal activity in the period important for change in the LH during the proestrus period of cyclic rats. The rats appeared to be healthy, and showed no anatomic pathological abnormality on the day of sampling.

3. Experimental design

3.1. Experiment 1: noradrenaline content and hormone profile

Forty-eight diestrus rats and those in persistent diestrus were decapitated (n = 8/group) for blood collection, removal and immediate freezing of their brains. Noradrenaline concentrations and GnRH content were measured in microdissections from the POA and MBH. NE, its metabolite and NE turnover rate, estimated by the MHPG/NE ratio, were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ED). Plasmatic concentration of the LH, FSH, estradiol and progesterone was determined by radioimmunoassay.

3.1.1. Brain microdissection

Coronal brain sections were obtained by using a cryostat at —15 °C (Microm® HM 505) according to the rat brain atlas (Paxinos and Watson, 2007). One section of 1500 μm and two consecutives sections of 1000 μm were obtained by microdissection by using the *punch* technique (Palkovits, 1973). The POA was microdissected from the first section (approximately +0.48 mm from the bregma until −1.08 mm), in one punch obtained with a 2.0 mm diameter needle, centered immediately above to the optic chiasm. The MBH was microdissected from the second and third sections, starting at approximately −1.8 and −2.8 mm from the bregma, respectively, in one punch bilaterally obtained with a 1 mm 'square puncher', centered on the third ventricle.

3.1.2. HPLC-ED

To analysis of concentrations of NE and metabolites in POA, by HPLC-ED, the samples were mixed with 100 μL of perchloric acid (PCA 0.15 M) and ethylene diaminetetraacetic acid (EDTA 0.1 mM) containing 10 pg/μL of isoproterenol (ISOP) as internal standard. The homogenate was centrifuged (13.000 rpm/5 min/4 °C), filtered (0.22 μm membrane; Durapore, Millipore) and hydrolyzed by heating to 94 °C for 5 min (Lookingland et al., 1991) before being injected into the HPLC-ED system by means of an autoinjector (SIL-10Advp; Shimadzu, Kyoto, Japan). Separation was performed at 35 °C in a 250 × 4-mm RP-18e column (Purospher, 5 μm; Merck, Darmstadt, Germany), preceded by a 4 × 4-mm RP-18e guard column (Lichrospher, 5 μm; Merck). The mobile phase consisted of 100 mM sodium dihydrogen phosphate, 15 mM sodium citratum, 10 mM sodium chloride, 0.1 mM EDTA, 0.4 mM sodium 1-octanesulphonic acid (Sigma-Aldrich) and 16% methanol (Omnisolv; EMD Chemical Inc., Gibbstown, NJ, USA) (pH 3.5). The pump (LC-10Advp; Shimadzu) flow rate was set at 0.4 mL/min, and the detector potential was 0.75 V (Decade; Antec, Leyden, The Netherlands). Chromatography data were plotted with the Class-VP software program (Shimadzu). Noradrenaline and MHPG were identified by their peak retention time and quantified by the internal-standard method based on the area under the peak. All samples from each brain area were measured in the same analysis. The intra-assay coefficient of variation was 0.96% for NE and 4.6% for MHPG. The NE level was considered to estimate the neurotransmitter contained in synaptic vesicles, whereas the MHPG level reflected the amount NE released in the sample (Lookingland et al., 1991). The MHPG/NE was used as a measure of neurotransmitter turnover. In the remaining pellet, the protein content was determined by the Bradford method (Bradford, 1976). The catecholamine content was expressed in pg/μg protein.

3.1.3. Radioimmunoassay

MBH samples were sonicated (50 μL HCl), and centrifuged (12,000 rpm/20 min/4 °C; Eppendorf®, Model 5417 R) to determine the GnRH content. Antibody GnRH R1245 was used, produced by Terry Nett (Department of Biomedical Sciences, Colorado State University, CO, USA); standard INC was supplied by Peninsula Laboratories (Bachem Inc., CA, USA), and hormone marked (^{125}I) by PerkinElmer (NEX-10 μCi 1630, PerkinElmer Life and Analytical Sciences, MA, USA). All dilutions were made in a phosphate buffer gel containing EDTA (pH 7.4), at 4 °C. The complex was precipitated with cold absolute ethanol and the lowest limit of detection was 0.24 pg/mL. The radioactivity of the precipitate was determined by using a gamma counter (Wizard 1470 Automatic Gamma Counter, Perkin Elmer) and the results were expressed as pg/mg protein in the MBH determined by protein assay (Bradford, 1976).

Plasma LH and FSH levels were determined by double-antibody RIA using the specific kit provided by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK, Bethesda, MD, USA). The antibodies used were anti-rat LH-S10 and FSH-S11 diluted with normal rabbit serum and standard preparation LH-RP3 and FSH-RP2 diluted in phosphate buffered gel 0.1% (0.01 M, pH = 7.5). The minimum detectable dose was 0.16 ng/mL for LH, and 0.09 ng/mL for FSH and the intra-assay coefficient of variation was 4%.

Progesterone was determined using a kit from MP Biomedicals LLC (Divisions Diagnostics, New York, USA); and for estradiol, the Siemens kit was used (Siemens Medical Solutions Dignostics, Los Angeles,

USA). The minimum detectable dose of estradiol (5.0 pg/mL) and progesterone (0.3 ng/mL) and the intra-assay value for estradiol (4.3%) and progesterone (7.6%) were calculated. All samples were assayed in duplicate and in the same assay to avoid inter-assay error.

3.2. Experiment 2: neuronal activity in the AVPV and noradrenergic neurons in the LC

Forty-eight experimental animals (n = 8/group) were anaesthetized with a mixture of ketamine (Cetamin®; Syntec, São Paulo, Brazil; 80 mg/kg body weight, i.p.) and xylazine (Xylazin®, Syntec; São Paulo, Brazil; 8 mg/kg body weight, i.p.) and perfused transcardially. The procedure was performed with phosphate-buffered saline (PBS) with heparin (5 IU/mL), followed by 4% paraformaldehyde in 0.1 M phosphate buffer and the brains were processed as previously described (Helena et al., 2006; Szawka et al., 2005). Frontal sections of 30 μm were cut in a cryostat approximately +0.48 mm to −0.48 from bregma for POA; and −9.36 mm to +10.32 mm from bregma for LC, according to the atlas of Paxinos and Watson (Paxinos and Watson, 2007). Sections were collected in four adjacent series and stored at −20 °C in culture dishes containing cryoprotectant solution (Watson et al., 1986) until the time of analysis. The sections were processed by immunohistochemistry for FRA (*Fos*) in the POA and FRA/TH in the LC. The number of FRA-ir and FRA/TH-ir neurons was analyzed bilaterally in the LC and AVPV as previously described (Leite et al., 2008).

3.2.1. Immunohistochemistry

Immunoperoxidase single labeling of FRA in sections from the AVPV, and double labeling of FRA/TH in sections from LC were performed as previously described (Helena et al., 2006; Leite et al., 2008; Szawka et al., 2005, 2013, 2009; Watson et al., 1986). After removal from the cryoprotectant solution, the sections were rinsed and incubated with anti-FRA rabbit antibody (K-25; Santa Cruz Biotechnology, Santa Cruz, CA, USA), at a dilution of 1:2000 for 40 h. After this, biotinylated anti-rabbit goat immunoglobulin G (BA-1000; Elite Kit, Vector Laboratories, Burlingame, CA, USA) at 1:600 for 90 min and avidin-biotin complex solution at a dilution of 1:100 for 1 h (Elite ABC kit, Vector Laboratories) were added. The antibody-peroxidase complex was visualized with a solution of nickel sulphate (25 mg/mL), 3,3′-diaminobenzidine-HCl (DAB 0.2 mg/mL) and H_2O_2 (1 μL/mL of 30% stock solution) in 0.175 M acetate buffer (pH 7.5). Sections were then incubated with anti-TH mouse antibody (anti-TH2; Sigma, Saint Louis, MO,USA) at a dilution of 1:500.000 for 40 h, followed by biotinylated horse anti-mouse horse immunoglobulin G (BA 2001; Vector Laboratories) at a dilution of 1:600 for 1 h and with Elite ABC kit for 1 h. TH was immunostained with a solution containing DAB (0.2 mg/mL) and H_2O_2 (1 μL/mL of 30% stock solution) in 0.05 M Tris–HCl buffer (pH 7.6). Sections were mounted on glass slides, cover slipped with Entellan (Merck, Darmstadt, Germany) and stored until the time of analysis of the groups.

To analyze the delimited area of AVPV, the rostral-caudal pattern was identified as previously described (Le et al., 2001). Immediately afterwards, the authors proceeded with bilateral counts of FRA-ir neurons in a square area of 19.200 μm^2 (240 × 716 pixels) and 28.800 μm^2 (240 × 956 pixels), corresponding to the coordinates 0.00 mm and −0.12 mm from bregma, respectively, using the third ventricle as the limit. In LC, double-labeled FRA/TH-ir neurons were bilaterally quantified, considering the coordinates −9.36 mm to −10.08 mm for all animals. The analyses were performed with an optical microscope (Axioskop 2 plus, Zeiss, Hallbergmoos, Germany) using the Axiovision 3.1 image analysis system (Zeiss).

4. Statistical analysis

According to the Shapiro-Wilk normality test, statistical differences were determined by two-way ANOVA using Sigma Plot v12.0 (SYSTAT, San Jose, CA, USA) followed by Newman-Keuls post-test for all the parametrical analyses. The relationship between the GnRH and NE content, as well as MHPG and estradiol were determined by means of the Spearman nonparametric correlation analysis. Mean values ± SEM are expressed in the graphs. The level of significance was set at $p < 0.05$ for all comparisons.

5. Results

To validate an appropriate model of reproductive senescence, the authors analyzed the changes occurring in the estrous cycle of the Wistar rats aged 18–20 months. They showed that initial change was marked by increased variability in the length of the estrous cycle phases, with diestrus lasting 12 days longer, similar to stage −2 in women, referred to as *early menopausal transition* (Harlow et al., 2012). This period

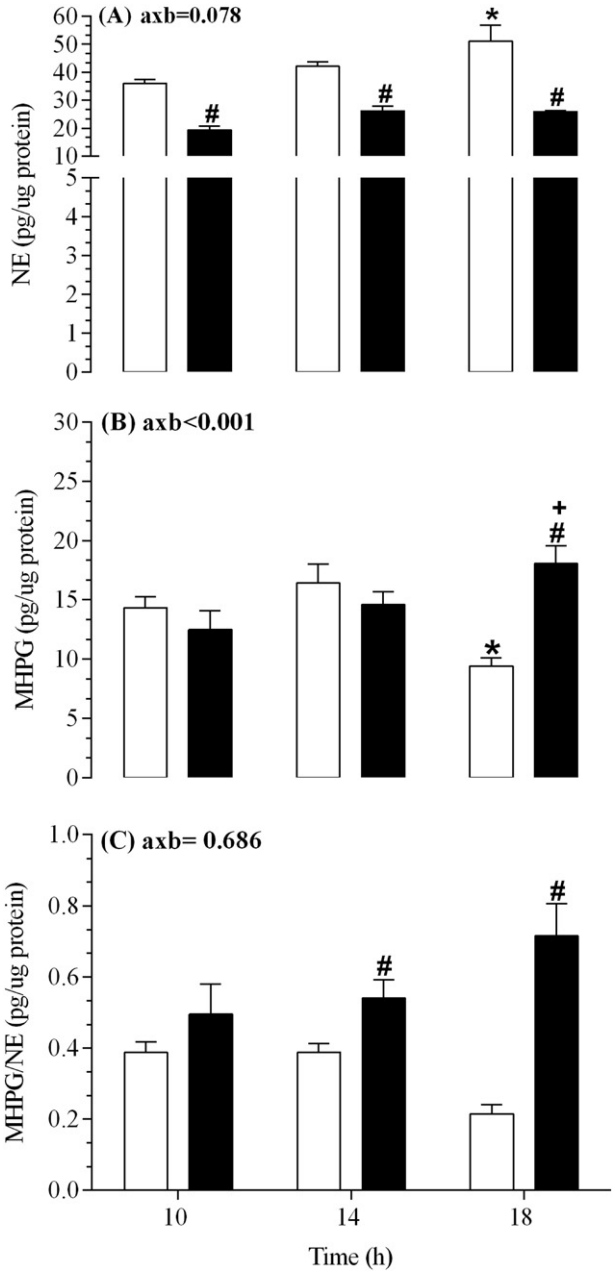
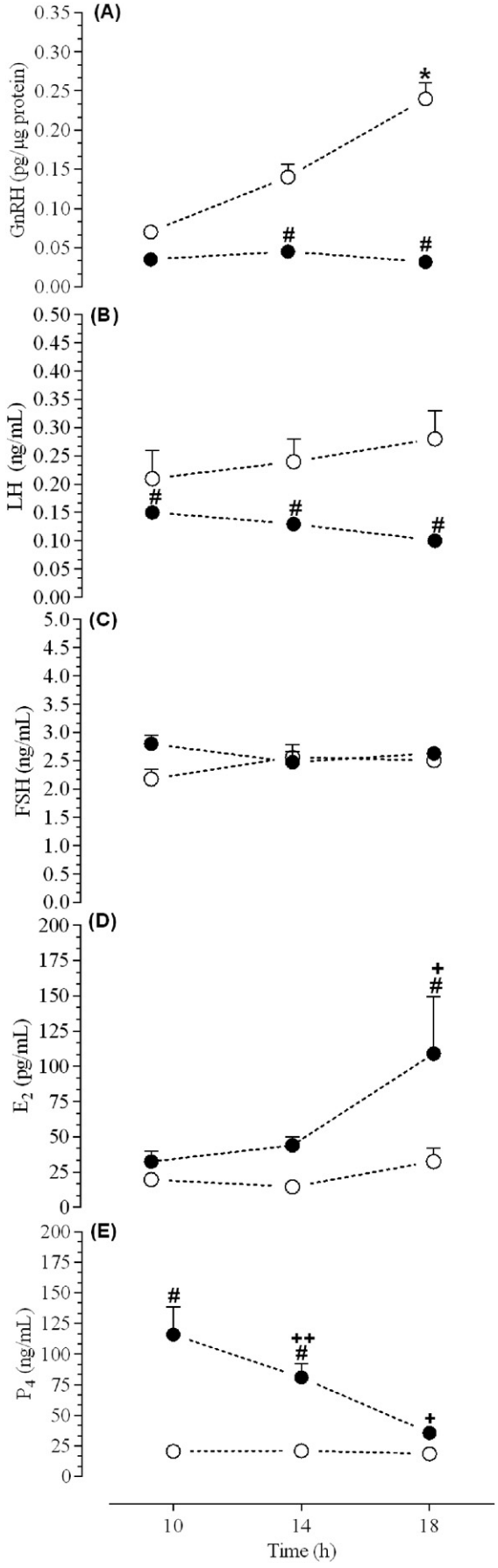


Fig. 1. The NE content (A), MHPG (B), MHPG/NE ratio (C), in the POA of adult-cyclic (□) and old-acyclic (■) rats at 10:00, 14:00, and 18:00 h in diestrous. The data expressed as the mean ± SEM. $^{\#}p < 0.05$ vs. cyclic rats at the same time interval; $^{*}p < 0.05$ vs. 10:00 h and 14:00 h/cyclic rat; $^{+}p < 0.05$ vs. 10:00 h and 14:00 h/acyclic rat; axb = interaction of age and time.



is defined as persistent diestrus with recurrence within 3 or 4 cycles with a variable length cycle. Transition early in the estrous cycle was also marked by decrease of the LH, characterizing the period of periestropause in these animals. Regularity of the estrous cycle was confirmed in the rats aged 4 months.

5.1. Noradrenaline content and hormone profile

These experiments evaluated the effect of age-related on neurotransmitter (NE); GnRH content; gonadotropins, and ovarian steroids of the female rats. Statistical analysis of the NEs and their main metabolite (MHPG) throughout the extension of the POA pointed out interaction between the factors age and time for the metabolite ($p < 0.001$). The NE content demonstrated that the level of these neurotransmitters (Fig. 1A) stored in the acyclic group was constant and lower than it was in the cyclic group ($p < 0.001$; $F_{(1,27)} = 126.012$), indicating a lower rate of NE synthesis in these animals. In the cyclic group an increased in this content was observed over time ($p_{10 \times 18\ h} < 0.001$ and $p_{14 \times 18h} < 0.05$).The MHPG metabolite content (Fig. 1B) showed significant differences at 18:00 h in both groups ($F_{(1,26)} = 2.697$), when intragroup comparisons ($p < 0.01$), and comparisons between cyclic and acyclic groups ($p < 0.001$) were made. In this time interval, the metabolite content was greater in older animals ($p < 0.05$). The release rate of the noradrenergic ratio MHPG/NE (Fig. 1C) was higher at 14:00 ($p < 0.05$) and 18:00 h ($p < 0.05$) in the acyclic group compared with the respective time intervals of the cyclic group ($F_{(1,24)} = 11.703$).

In the group of the cyclic rats, the GnRH content increased significantly at 18 h ($p_{10x18h} < 0.01$), and the plasma concentration of LH and FSH did not change during the same period (Fig. 2A, B and C). No effects of time of day were found in persistent diestrous rats as regards the GnRH content in the MBH, and LH and FSH plasma levels. However, in comparison with adult rats, the MBH GnRH content in the old rats was lower at 14:00 h ($p < 0.05$) and 18:00 h ($p < 0.001$; $F_{(1,24)} = 21.104$) and LH plasma levels were lower in all time intervals studied ($p < 0.05$ at 10:00 and 14:00 h; $p < 0.001$ at 18:00 h; $F_{(10,230)} = 29.736$). The interaction between the factors age and time considering the hormonal parameters proved to be significant for the GnRH ($p < 0.05$). Furthermore, no differences were observed between FSH of the adult and old rats ($p = 0.134$; $F_{(1,28)} = 1.877$).

Fig. 2D shows the plasma estradiol levels as measured by RIA for intact female Wistar rats. A significant effect of time of day was observed, owing to significantly higher estradiol levels at 18:00 h than at 10:00 h ($p < 0.01$) and 14:00 h ($p < 0.05$) in acyclic rats. There was no effect of time of day on estradiol levels in these cycling animals, or any interaction of time of day with cycling status or age. Post-hoc analysis indicated that estradiol levels were significantly higher at 18:00 h in persistent diestrus than in diestrous rats ($p < 0.05$). A significant effect of age on plasma progesterone levels was observed with significantly higher levels in acyclic than in cyclic rats ($p < 0.001$). In cycling (diestrus) rats, no significant effect of age and time of day on progesterone levels were observed (Fig. 2E). In acyclic rats (persistent diestrus), a significant effect of time of day was observed, owing to significantly higher progesterone levels at 10:00 h ($p < 0.001$) and 14:00 h ($p < 0.01$) than at 18:00 h. Post-hoc analysis indicated that progesterone levels were significantly higher in persistent diestrus than in diestrous rats ($p < 0.05$). The authors point out that ovarian steroids in acyclic rats were inversely proportional to each other (Fig. 2D and E), i.e. the estradiol levels increased while the progesterone levels decreased during the course of the time intervals studied on the persistent diestrus day.

Fig. 2. The hypothalamic GnRH content (A), plasma levels of gonadotropins (B and C) and sex-steroid levels (D and E) of adult-cyclic (○) and old-acyclic (●) rats. All the data are expressed as the mean ± SEM. $^{\#}p < 0.05$ vs. cyclic rats at the same time interval; $^{*}p < 0.05$ vs. 10:00 h and 14:00 h/cyclic rat; $^{+}p < 0.05$ vs. 10:00 and 14:00 h/acyclic rat; $^{++}p < 0.05$ vs. 10:00 h/acyclic rat; axb = interaction of age and time.

The variables for NE (POA) and GnRH (MBH) content, as well as MHPG/NE (POA) and plasma E_2 for both adult and old rats, revealed a positive correlation (Fig. 3A $r_S = 0.74$ and 3C; $r_S = 0.4866$; $p < 0.0001$). However, comparisons between MHPG/NE and LH plasmatic were negatively correlated (Fig. 3B; $r_S = -0.6311$; $p < 0.001$).

5.2. Neuronal activity in the AVPV and noradrenergic neurons in the LC

This purpose of this experiment was to determine the specific activity of AVPV neurons and noradrenergic neurons in the LC during the aging of the female rats. The level of FRA-ir expression in the AVPV neurons of acyclic rats was higher at 14 and 18:00 h ($p < 0.001$; $F_{(1,24)} = 71.608$) when compared with the same time intervals in cyclic rats ($p < 0.001$). No difference was observed between cyclic rats, as shown in Fig. 4G. Illustrative photomicrographs of nuclear-labeling of Fos-related antigen (FRA) in the AVPV (Fig 4A) of cyclic rats (Fig. 4B–C) perfused at 18:00 h, demonstrating less intense FRA activation of AVPV neurons when compared with acyclic rats (Fig. 4D–F). Fig. 5 shows FRA/TH-ir expression by neurons in LC in the diestrus phase. Double-labeled cells were identified as those that exhibited brown cytoplasmic stain and a black nucleus. The results (Fig.5G) demonstrated a higher level of expression at 10 and 14:00 h ($p < 0.05$; $F_{(1,26)} = 23.763$) in acyclic rats when compared with same time intervals in cyclic rats. In this analysis, there was no interaction between the factors age and time ($p = 0.498$). Fig. 5A–C and D–F display representative photomicrographs of FRA/TH-ir neurons in the LC of cyclic and acyclic rats, respectively, perfused at 10:00 h in diestrus.

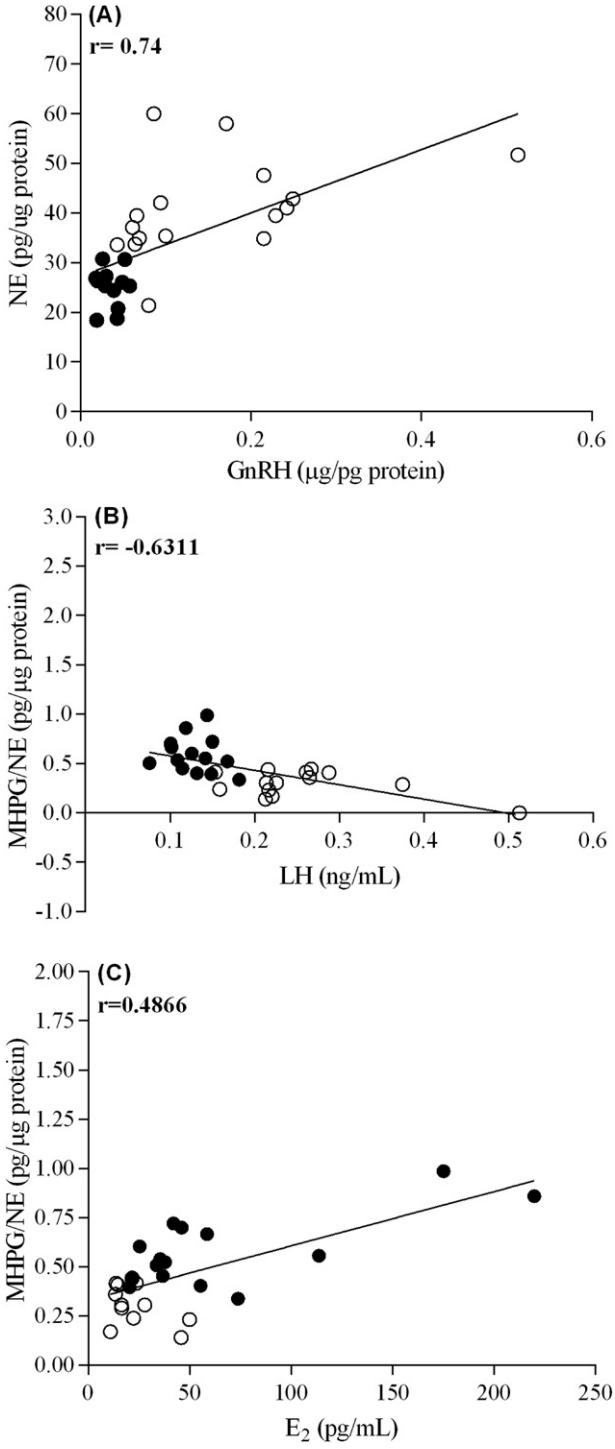


Fig. 3. Spearman nonparametric correlations analysis between neurotransmitter, GnRH, LH and E_2 of adult-cyclic (○) and old-acyclic (●) rats. (A and C) Positive and (B) negative correlations. r = correlation coefficient.

6. Discussion

This study demonstrated that NE plays a role in regulating the neuroendocrine status present in acyclic rats. The authors' results showed age-related increase in the activity of neurons in the LC and POA/AVPV, and in the turnover of NE, but decrease in content of NE, GnRH and plasma concentration of LH in acyclic female rats. However, in adult cyclic rats, they found an increase in the NE and GnRH content in POA/AVPV, indicating involvement of this neurotransmitter in the neuroendocrine events to induce the pre-ovulatory LH surge and ovulation.

In the reproductive cycles of rats, the importance of NE in inducing the LH surge was clearly established. There was evidence of direct action, mediated by both α1 and β- adrenergic receptors in the soma and/or dendrites, and of indirect action of NE on releasing hormone-containing neurons (Han and Herbison, 2008; Hosny and Jennes, 1998; Szawka et al., 2013). Noradrenergic involvement of the brainstem neuron groups located in A1/A2 (Herbison, 1997; Ibrahim and Briski, 2014) and A6 (Anselmo-Franci et al., 1997, 1999; Berridge and Waterhouse, 2003; Wang et al., 2015) to hypothalamus neurons has been demonstrated to be a source and trigger of the GnRH/LH-mediated pre-ovulatory surge increased by gonadal steroids. The role of NE in reproductive-aged rodents is evident, and is associated with ovarian steroids in regulating the secretion of hypothalamic GnRH through neurotransmitters, and with the neural events that induce the LH surge (Helena et al., 2006). Furthermore, estradiol and progestin receptors in LC neurons suggest that these cells are responsive to variations in circulating levels of ovarian steroids (Szawka et al., 2009). The authors' results showed the actions previously described (Sellix and Menaker, 2011) with regard to effects of gonadal steroids on gonadotropin secretion in estrous cyclicity. In cyclic rats, the diestrus phase is characterized by constant concentrations of LH (Fig. 2B) and gonadal steroids (Fig. 2D-E), over the course of the time intervals analyzed in accordance with the present study.

There is evidence that aging of the neuroendocrine components of the reproductive axis in women is characterized by a decrease in the frequency of pulsatile GnRH (Hall et al., 2000) and pituitary responsiveness (Shaw et al., 2009). In the late 80s, a study in rodents, (Rubin and Bridges, 1989) showed that in old females, there was a lower volume in the Golgi apparatus and rough endoplasmic reticulum in the GnRH neurons; some years later, changes were shown in the morphology, cytoarchitecture, and ultrastructure of GnRH perikarya and terminal (Yin et al., 2009). In the present study, the authors showed a lower GnRH content and plasma LH level (Fig. 2A, B), but the level of gonadal steroids was higher in persistent diestrous (Fig. 2D, E) when compared with that of adult rats on the diestrous day. These results suggest that the negative feedback of estradiol prevailed in the GnRH neuron and indicated that biosynthetic capacity of gonadal was maintained for a

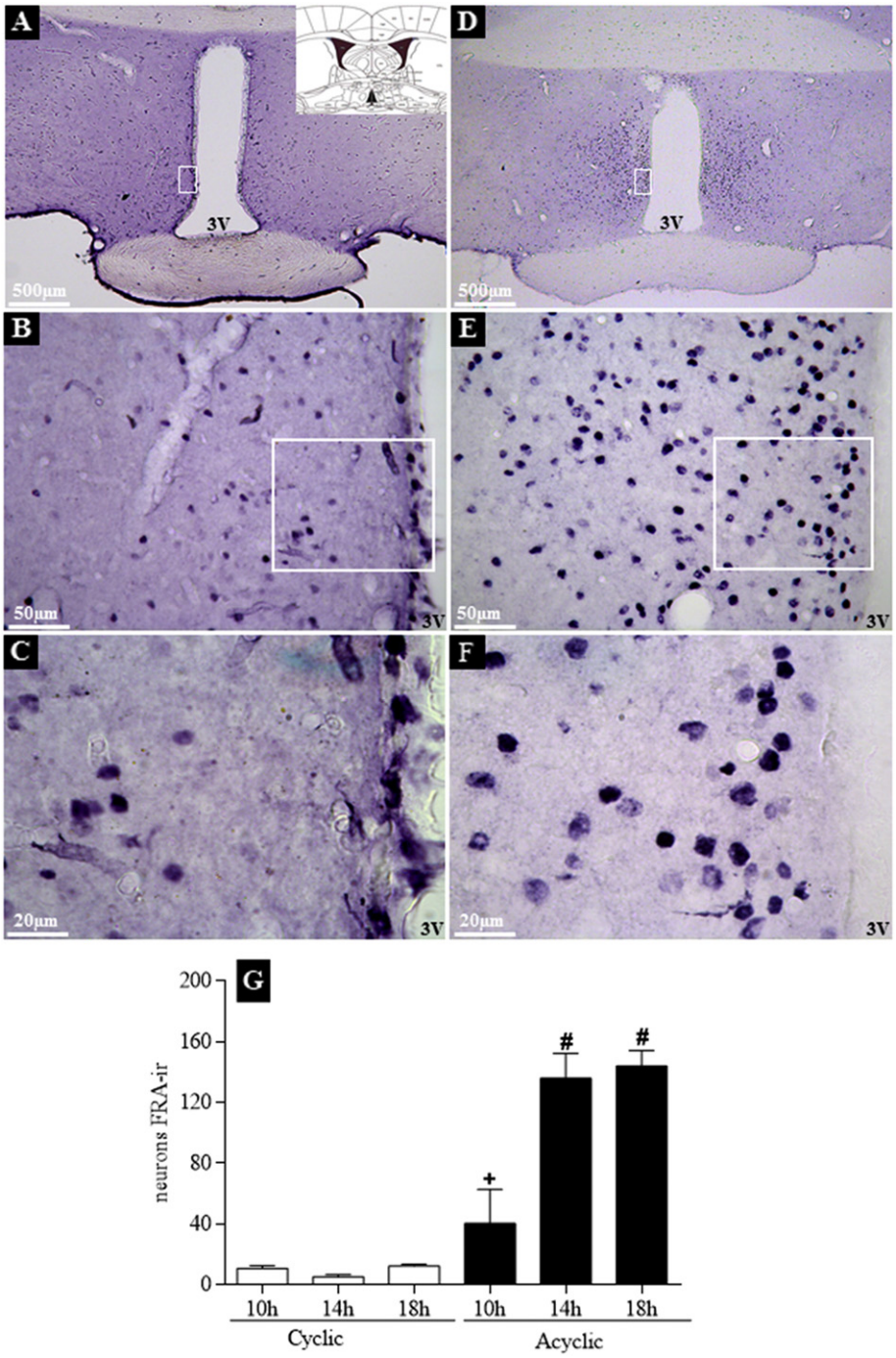


Fig. 4. Representative photomicrographs of the nuclear labeling of FRA in the AVPV (bregma 0.00 mm) of adult-cyclic (A–C) and old-acyclic (D–F) rats perfused in diestrous. 3 V = third ventricle. The scale bar for AVPV = 500 μm (50× magnification), 50 μm (400× magnification), and 20 μm (1000× magnification). (G) Graphic representation of higher neuronal activity observed in old-acyclic (■) compared to adult-cyclic (□) rats. The mean ± SEM number of FRA neurons are expressed in the graph. $^{\#}p < 0.05$ vs. cyclic rats at the same time interval; $^{+}p < 0.05$ vs. 14:00 and 18:00 h/acyclic rat.

long period during aging. It is interesting that the neurophysiological pathways responsible for generating the LH surge is dependent on endogenous ovarian steroids and these exert their effects on monoaminergic systems, changing the levels of enzymes that synthesize and degrade NE (Donner and Handa, 2009). Levels of monoamine reuptake transporters and receptors (Le Saux et al., 2006; Osterlund et al., 2000) and coupling of receptors to intracellular second messenger systems are also influenced by estradiol (Mize and Alper, 2002; Mize et al., 2001). It is important to point out that in young and adult rats, estradiol levels are higher than they are in old rats and the effect of aging on estrogen negative feedback on LH is true across a range of low level of E2 (Shaw et al., 2011). In this study, the authors could observe that the configuration of graphs quantifying the activity of AVPV and LC neurons was similar to that of the plasma concentration of E2 and progesterone, respectively. Thus, the authors suggest that the increase in plasma steroid levels contributed to the high activity of AVPV and LC neurons and NE turnover rate during the persistent diestrus, but these neurons were less effective in the synthesis and storage of the neurotransmitters required for increasing GnRH and LH (Figs. 1–2). Furthermore, these results suggested that the properties of cells, such as the ability to synthesize, store and release GnRH were compromised and contributed to changes in the temporal pattern of neuroendocrine signaling, thereby

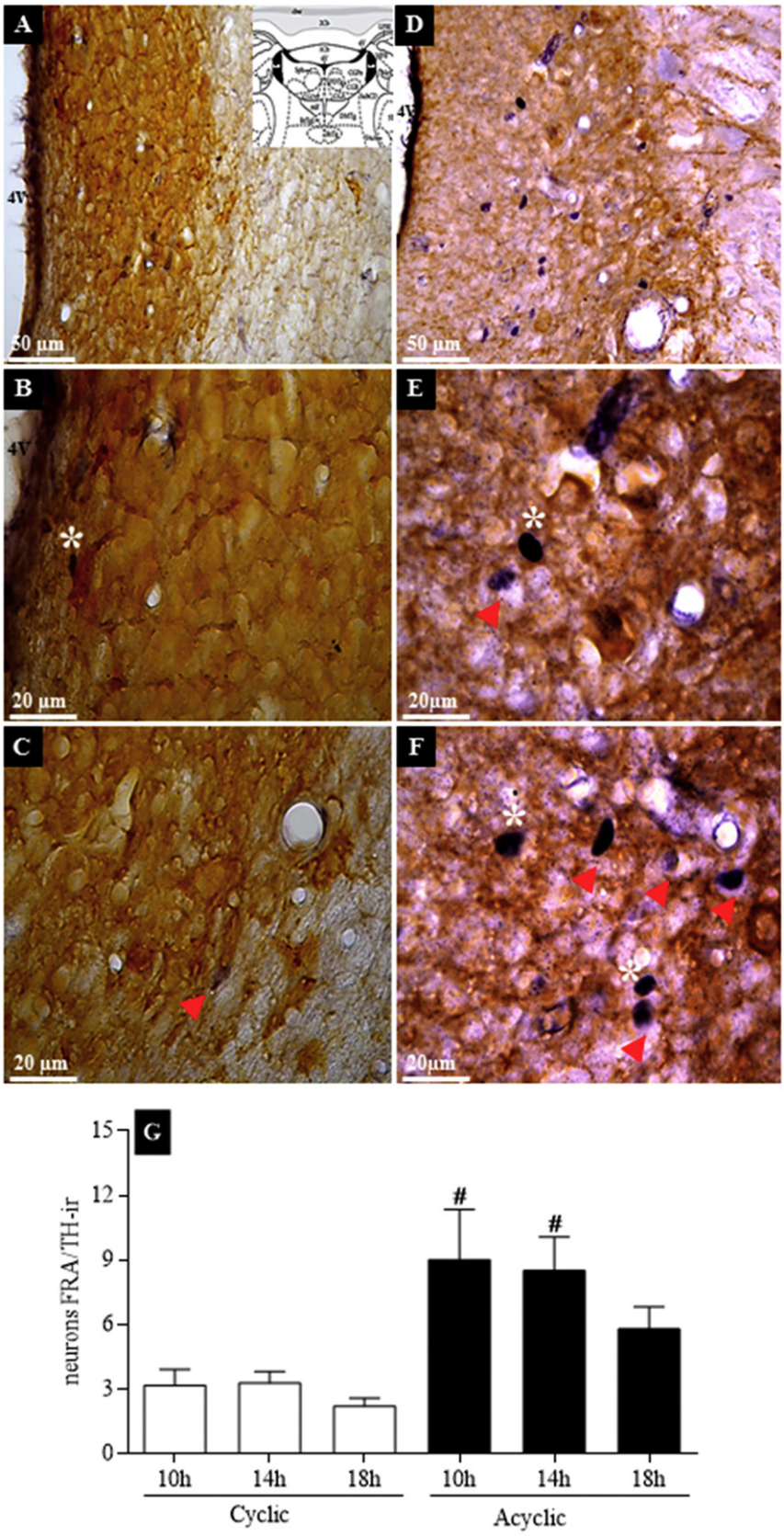


Fig. 5. Representative photomicrographs of the number of FRA/TH-ir neurons in the locus coeruleus (LC). Adult-cyclic (A–C) and old-acyclic (D–F) rats were perfused in diestrous. 4V = fourth ventricle. The scale bar for LC = 50 µm (400× magnification) and 20 µm (1000× magnification). * indicates double-labeled neurons (TH staining is cytoplasmic; FRA is nuclear) and arrows indicate labeling for FRA only. (G) Graphic representation of higher LC neuronal activity observed in old-acyclic (■) compared to adult-cyclic (□) rats. The mean ± SEM number FRA/TH-ir neurons are expressed in the graph. $^{\#}p < 0.05$ vs. cyclic rats at the same time interval.

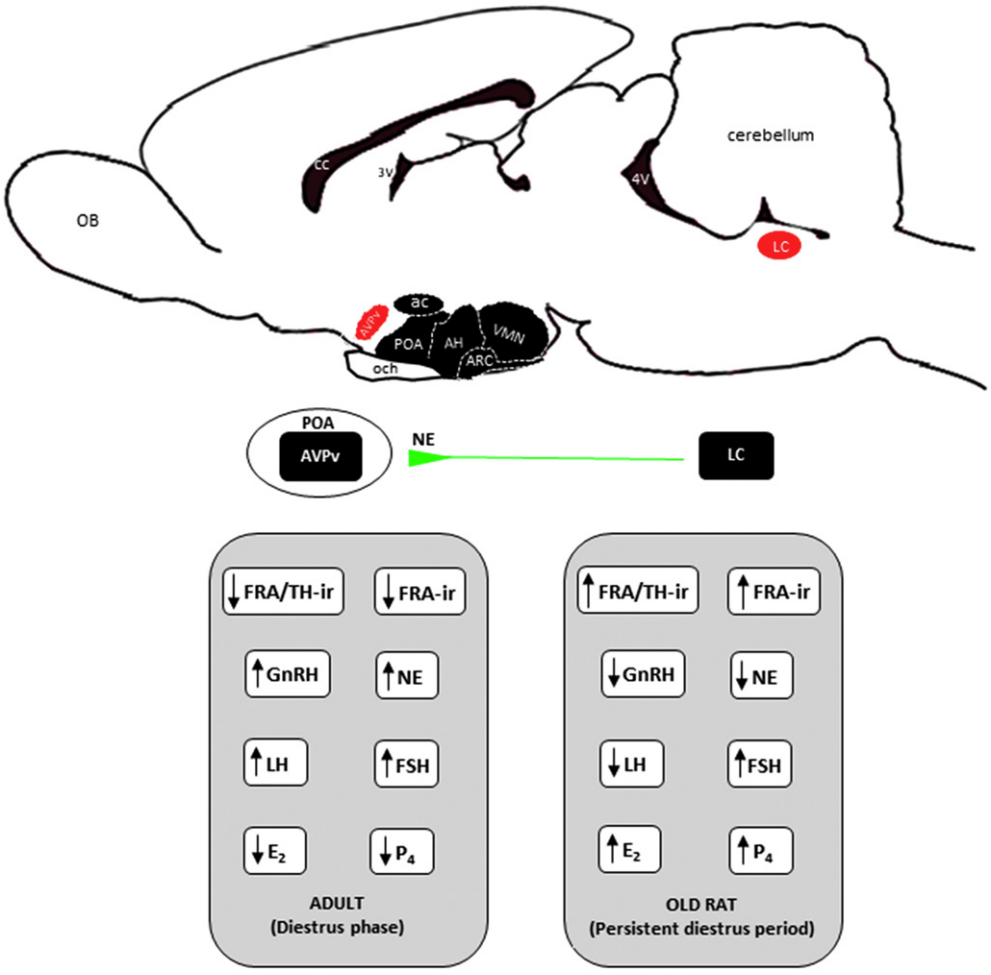


Fig. 6. Schematic diagram showing results obtained in this study com adult-cyclic and old-acyclic rats. Brain regions and connections provide a map of the neural circuits and a neurobiological basis for the array of that can emerge during periestropause.

compromising the accuracy of inhibitory and stimulatory effects, causing irregularity in the estrous cycle and determining reproductive senescence.

Studies in postmenopausal women have analyzed pituitary response to GnRH as a function of aging, measuring the amplitude of the pulsatile secretion of LH or exogenous pituitary GnRH response. The decrease in LH pulse amplitude with aging has been documented in some studies (Genazzani et al., 1997; Hall et al., 2000), but it has not been confirmed (Lambalk et al., 1997). It is of interest that the decrease in gonadotropin levels in older compared with adults rats was significant for LH but not for FSH (Fig. 2B and C), in the present study. The authors' results indicated that LH levels reflected a balance between pituitary responsiveness and the hypothalamic GnRH, suggesting that LH secretion was more dependent on GnRH than FSH was. Maybe the FSH levels more closely reflect the effect of aging on the pituitary itself in comparison with LH whose regulation is more strongly influenced by the complex changes in GnRH secretion (Fig. 6).

In summary, the data of the present study suggest that higher level of activity of the neurons of POA/AVPV and NE neurons of LC in acyclic rats, associated with the lower capacity for the synthesis and storage of neurotransmitters and neurohormones compromises the signaling necessary for the occurrence of maximum secretion of LH and subsequent ovulation, characterizing the process of reproductive senescence in female rodent.

Disclosure statement

None of the authors have any actual or potential conflicts to declare.

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