Effects of Estrogen on Growth Hormone Pulsatility in Peripheral Blood and Neuropeptide Profiles in the Cerebrospinal Fluid of Goats

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Abstract. We previously reported that growth hormone (GH) pulses were negatively associated with neuropeptide Y (NPY) profiles in cerebrospinal fluid (CSF) of the third ventricle of Shiba goats. In addition, while most GH pulses were coincident with GH-releasing hormone (GHRH) pulses, there was no correlation between GH and somatostatin (SRIF) levels. The present study was performed to elucidate the relationship between GH pulses and these neuropeptide levels in CSF when estradiol (1.0 mg/head) was subcutaneously administered to ovariectomized goats. CSF and plasma samples were collected every 15 min for 18 h (from 6 h before to 12 h after injection). GH levels in peripheral blood and GHRH, SRIF and NPY levels in CSF were measured by radioimmunoassay. Pulse/trough characteristics and correlations were assessed by the ULTRA algorithm and cross-correlation analysis. Before estradiol was injected, significant coincidence was found between GHRH pulses and GH pulses, and negative coincidence was found between NPY troughs and GH pulses. Six to 12 h after estradiol injection, the amplitude and area under the curve (AUC) of the GH pulses were markedly increased. The duration and AUC of the GHRH pulses in the CSF were also increased, and stronger synchrony of GHRH with GH was observed. In contrast, the baseline of NPY was significantly decreased, and the negative correlation between the GH pulses and NPY troughs disappeared. The parameters of SRIF troughs were not clearly changed. These observations suggest that estrogen enhances the pattern of secretion of GH in the goat via enhancement of GHRH pulses and decrease of NPY levels.

Key words: Estrogen, Growth hormone, Growth hormone-releasing hormone, Neuropeptide Y, Somatostatin

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[26], has been reported to inhibit GH secretion [27, 28]. In our study [22], strong correlations were found neither between GH and GHRH nor GH and SRIF. However, NPY levels in CSF fluctuated episodically at around two-hour intervals, and NPY troughs were robustly synchronized with GH pulses [22]. In addition, it is reported that NPY is released into the hypophysial-portal circulation in rats [29], and NPY Y1 receptors are expressed in the pituitary to suppress GH secretion. Taken together, it is suggested that periodic decrease in NPY may be involved in the generation of GH pulsatility in goats.

In the present study, we examined the effects of estrogen on GH levels in peripheral blood and GHRH, SRIF and NPY levels in CSF. To determine the interrelationships between GH and neuropeptides with precision, pulse/trough characteristics and correlations of secretory profiles were assessed using two independent mathematical approaches.

Materials and Methods

Animals

Three adult Shiba goats (2–4 years of age) ovariectomized at least six months before the experiments were used in this study. They were maintained for experimental purposes in a closed colony at the Experimental Station for Bio-Animal Science of the University of Tokyo. They were loosely restrained indoors by being tied to stanchions and kept at a temperature of 23°C with lights on from 0600 to 1800 h (12L–12D). To avoid effects of grain feeding on the GH secretory pattern [31], only alfalfa hay was given from at least a week before the experiments. During the experiments, they were allowed free access to food (alfalfa hay) and water. All experiments were conducted according to the guidelines for the care and use of laboratory animals, Graduate School of Agriculture and Life Sciences, The University of Tokyo.

Surgical procedure

At least two months before the experiment, a stainless steel cannula was stereotaxically introduced into the third ventricle as previously reported [20]. Briefly, the goat was anesthetized with isoflurane and positioned in a stereotaxic frame (Narishige Scientific Instrument Laboratory, Tokyo, Japan). A 22-gauge spinal needle (Terumo, Tokyo, Japan) was inserted into the lateral ventricle (4 mm lateral and 5 mm posterior to bregma, and 17 mm beneath the dura), and 500 μl of radio-opaque liquid (Omnipaque, Daiichi Pharmaceutical, Tokyo, Japan) was injected. A lateral X-ray was taken, yielding specific landmarks of the ventricular system. The cannula was approximately 50 mm in length and was made from an 18-gauge spinal needle blunted at both ends. A 21-gauge stylet was inserted into the cannula to penetrate the tissue and to prevent CSF backflow. The cannula and stylet were stereotaxically lowered through the center of the sagittal sinus and aimed slightly anterior to the vertical tangent of the interthalamic adhesion and about 10 mm below a horizontal line drawn though the middle of the interventricular foramen. The position of the cannula was finally confirmed by an additional lateral X-ray, which was superimposed on the first X-ray, and the cannula was immobilized by filling the burr hole with acrylic dental cement. A plastic cap with a screw-off top was centered over the cannula and anchored to the skull with stainless steel screws and acrylic dental cement.

Sampling of blood and CSF

When a goat was used in the experiment, the top of the protective cap was removed and the stylet was pulled out. A silastic tube (inside diameter, 0.5 mm; outside diameter, 0.8 mm; length, 30 cm) filled with sterile saline was attached to the cannula with a 1 cm silastic collar, in which the proximal end of the collecting tubing was glued with an adhesive. Then, the plug was removed, and the CSF that overflowed spontaneously from the distal end of the collecting tube was collected. At each sampling, approximately 200 μl of CSF was collected after discarding the dead space (60 μl). CSF sampling was performed at 15-min intervals for 18 h in this study (starting at 0600 h). Animals were subcutaneously administered 1 mg of estradiol (Sigma-Aldrich, St. Louis, MO, USA) dissolved in sesame oil at 1200 h. In our preliminary data, the estrogen levels at 3 and 18 h after injection were 58.3 ± 16.3 pg/ml and 5.0 ± 1.4 pg/ml (n=3, mean ± SE), respectively, which was comparable to those during follicular phase [5]. During the sampling period, the goats were loosely restrained by being tied to stanchions. The samples were stored at −20°C until the assays for GHRH, SRIF and NPY were carried out. Peripheral blood samples (1 ml) were collected at the same time as CSF sampling through an indwelling jugular catheter (Argyle Medical Catheter, 18 gauge and 70 cm in length, Nippon Sherwood Medical Industries, Tokyo, Japan). Blood samples were placed in tubes containing heparin. After centrifugation, plasma was separated and stored at −20°C until assayed for GH.

Radioimmunoassays

To determine the concentrations of GH, GHRH, SRIF and NPY, radioimmunoassay (RIA) methods were used as previously reported [20]. Plasma GH concentrations were determined by double-antibody RIA using anti-bovine GH (bGH) antiserum. The bGH standard preparation and the hormone for iodination were previously reported [20]. The goat was anesthetized with isoﬂurane and positioned in a stereotaxic frame (Narishige Scientiﬁc Instrument Laboratory, Tokyo, Japan). A 22-gauge spinal needle (Terumo, Tokyo, Japan) was inserted into the lateral ventricle (4 mm lateral and 5 mm posterior to bregma, and 17 mm beneath the dura), and 500 μl of radio-opaque liquid (Omnipaque, Daiichi Pharmaceutical, Tokyo, Japan) was injected. A lateral X-ray was taken, yielding specific landmarks of the ventricular system. The cannula was approximately 50 mm in length and was made from an 18-gauge spinal needle blunted at both ends. A 21-gauge stylet was inserted into the cannula to penetrate the tissue and to prevent CSF backflow. The cannula and stylet were stereotaxically lowered through the center of the sagittal sinus and aimed slightly anterior to the vertical tangent of the interthalamic adhesion and about 10 mm below a horizontal line drawn though the middle of the interventricular foramen. The position of the cannula was finally confirmed by an additional lateral X-ray, which was superimposed on the first X-ray, and the cannula was immobilized by filling the burr hole with acrylic dental cement. A plastic cap with a screw-off top was centered over the cannula and anchored to the skull with stainless steel screws and acrylic dental cement.
GH and GHRH pulses and NPY troughs were identified by means of the ULTRA algorithm using the coefficient values calculated from the intra-assay CVs for each corresponding peptide [19, 33]. Time-series data were separated into three periods; preinjection, 0 to 6 h after injection and 6–12 h after injection. For each pulse/trough, the heights above/under the calculated baseline (amplitude) and the duration were determined. The frequency was calculated by measuring the interval between two peaks. The AUC was also assessed for each pulse. To analyze fluctuations in the pulse/trough profiles during these periods, Tukey-Kramer’s post-hoc test was used for multiple comparisons. Coincidence between a GH pulse and neuropeptide pulse/trough was evaluated as described previously [17, 22, 34]. Random data for GH pulse and neuropeptide pulse/trough series obtained with Monte Carlo shuffling (n=1,000) were used as a control. Significance was evaluated using the paired-t test.

**Cross-correlation analysis**

Cross-correlation analysis was performed with a method described previously [22]. Pearson correlation analysis was performed using GH values paired to the corresponding lagging neuropeptide values using various lag-times. A positive (negative) lag-interval corresponded to a neuropeptide measurement time following (preceding) the GH measurement time. Pearson coefficients, transformed to Z-scores, were tested against the null hypothesis of absence of correlation using the paired-t test [35]. The level of significance for cross-correlation was set at a protected value of P<0.05. The analysis was performed over a range of –45 to +45 min between the two series.

**Results**

**Pulse/trough coincidence analysis**

All the time-series data of these peptides are shown in Fig. 1. Table 1 presents all the pulse/trough profiles of GHRH, SRIF, NPY and GH using the ULTRA algorithm for every 6-h period (Prein-
6 h), and these increases continued in the second period after injection and 0–6 h after injection. The frequency, duration and baseline of the GH pulses were significantly increased from the first period (0–6 h) and these increases continued in the second period after injection (6–12 h). None of the parameters of the SRIF troughs were significantly changed. Interestingly, the baseline of NPY was drastically decreased during 6–12 h after injection.

The concordance between GH pulses and each neuropeptide pulse/trough was examined as described previously [22]. Table 2 indicates the likelihood that neuropeptide pulses/troughs exist within the time series in the −30 to +30 min lag for the GH pulse peak. The likelihoods that the GH pulse existed within the time series in the −30 to +30 min lag for neuropeptide pulses/troughs were also calculated. The concordance ratio was compared to random shuffled peak time series as controls. Before estradiol was injected, GH pulses were associated with GHRH pulses, but GHRH pulses were not strongly associated with GH pulses. After estradiol injection, although the coincidence rate values of the GHRH pulses and GH pulses were decreased, significant synchronicity of GHRH pulses to GH pulses appeared (P=0.044 during 0–6 h after injection and P=0.003 during 6–12 h after injection). Significant synchronicity of GH pulses to GHRH pulses was also observed until 6–12 h after injection. SRIF troughs were highly synchronized with GH

### Table 1. Effects of estradiol on pulse/trough profiles of GH and neuropeptides (NPs)

<table>
<thead>
<tr>
<th></th>
<th>Amplitude (pg/ml for NPs/ ng/ml for GH)</th>
<th>Frequency (h)</th>
<th>Duration (min)</th>
<th>Baseline (pg/ml for NPs/ ng/ml for GH)</th>
<th>AUC (pg/ml/pulse for NPs/ ng/ml/pulse for GH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHRH pulse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre (10)</td>
<td>11.8 ± 1.9</td>
<td>1.44 ± 0.32</td>
<td>30.0 ± 3.9</td>
<td>7.7 ± 0.6</td>
<td>15.8 ± 2.4</td>
</tr>
<tr>
<td>0–6 h (10)</td>
<td>17.2 ± 4.4</td>
<td>1.72 ± 0.26</td>
<td>56.3 ± 8.9*</td>
<td>7.2 ± 1.1</td>
<td>59.6 ± 18.1*</td>
</tr>
<tr>
<td>6–12 h (8)</td>
<td>13.3 ± 2.5</td>
<td>2.18 ± 0.43</td>
<td>56.3 ± 10.9*</td>
<td>8.3 ± 1.3</td>
<td>33.0 ± 10.3*</td>
</tr>
<tr>
<td>SRIF trough</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pre (11)</td>
<td>51.1 ± 10.8</td>
<td>1.19 ± 0.18</td>
<td>38.2 ± 4.2</td>
<td>101.9 ± 11.1</td>
<td></td>
</tr>
<tr>
<td>0–6 h (9)</td>
<td>33.4 ± 5.6</td>
<td>1.81 ± 0.37</td>
<td>46.7 ± 7.7</td>
<td>104.2 ± 14.5</td>
<td></td>
</tr>
<tr>
<td>6–12 h (11)</td>
<td>51.8 ± 14.6</td>
<td>1.77 ± 0.24</td>
<td>38.2 ± 5.1</td>
<td>128.2 ± 15.8</td>
<td></td>
</tr>
<tr>
<td>NPY trough</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre (6)</td>
<td>90.6 ± 20.7</td>
<td>1.60 ± 0.34</td>
<td>37.5 ± 8.4</td>
<td>644.3 ± 10.9</td>
<td></td>
</tr>
<tr>
<td>0–6 h (8)</td>
<td>124.5 ± 26.7</td>
<td>3.13 ± 0.46</td>
<td>41.3 ± 8.4</td>
<td>607.7 ± 30.7</td>
<td></td>
</tr>
<tr>
<td>6–12 h (11)</td>
<td>187.3 ± 41.7*</td>
<td>1.72 ± 0.29</td>
<td>37.5 ± 5.7</td>
<td>466.0 ± 44.6*</td>
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<tr>
<td>GH pulse</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pre (7)</td>
<td>14.4 ± 8.0</td>
<td>2.08 ± 0.45</td>
<td>42.9 ± 6.1</td>
<td>1.6 ± 0.2</td>
<td>15.5 ± 4.9</td>
</tr>
<tr>
<td>0–6 h (3)</td>
<td>20.7 ± 13.0</td>
<td>4.33 ± 0.98</td>
<td>35.0 ± 10.0</td>
<td>1.4 ± 0.1</td>
<td>47.7 ± 35.4</td>
</tr>
<tr>
<td>6–12 h (7)</td>
<td>56.8 ± 14.9*</td>
<td>3.86 ± 1.50</td>
<td>53.6 ± 6.4</td>
<td>1.3 ± 0.1</td>
<td>90.4 ± 29.3*</td>
</tr>
</tbody>
</table>

Pre: preinjection period, 0–6 h: 0 to 6 h after injection, 6–12 h: 6 to 12 h after injection. AUC: area under the curve. Values in parentheses are numbers of detected pulses/troughs. Other values are means ± SEM. Symbols indicate significant differences compared with Pre (* and #: P<0.05 and P<0.1, respectively).

### Table 2. Effects of estradiol on concordances between GH and each neuropeptide (NP)

<table>
<thead>
<tr>
<th>NP</th>
<th>GH pulse associated with NP</th>
<th>NP associated with GH pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed (%)</td>
<td>Randomized (%)</td>
</tr>
<tr>
<td>GHRH pulse</td>
<td>Pre</td>
<td>100.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>0–6 h</td>
<td>100.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>6–12 h</td>
<td>72.2 ± 14.7</td>
</tr>
<tr>
<td>SRIF trough</td>
<td>Pre</td>
<td>55.6 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>0–6 h</td>
<td>100.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>6–12 h</td>
<td>61.1 ± 20.0</td>
</tr>
<tr>
<td>NPY trough</td>
<td>Pre</td>
<td>88.9 ± 11.1</td>
</tr>
<tr>
<td></td>
<td>0–6 h</td>
<td>33.3 ± 33.3</td>
</tr>
<tr>
<td></td>
<td>6–12 h</td>
<td>44.4 ± 29.4</td>
</tr>
</tbody>
</table>

Pre: preinjection period, 0–6 h: 0 to 6 h after injection, 6–12 h: 6 to 12 h after injection. Tolerance ranges of lags were ±30 min. Values are means ± SEM. The observed data were compared with random pulses/troughs estimated by Monte Carlo shuffling using the paired t-test.
pulses only 0–6 h after injection, but there were no other significant changes between the GH pulses and SRIF troughs. NPY troughs were negatively synchronized with GH pulses during the preinjection period (P=0.007). After estradiol injection, the negative coincidences between GH and NPY disappeared (P=0.539 during 0–6 h after injection and P=0.511 during the 6–12 h after injection). There was no significant synchronicity in pulses/troughs among the neuropeptides measured in this study (data not shown).

Cross-correlation analysis
Cross-correlation analysis was applied as a time lag-specific test of linear relationships between plasma GH concentrations and neuropeptide levels in the CSF. Fig. 2 shows individual cross-correlation plots of GH and GHRH/SRIF/VPY for every 6-h period. Before estradiol was injected, a significant negative correlation was found between NPY values and GH values for the 15 to 30 min time lag. However, these correlations disappeared after estradiol injection. In contrast, although there was no correlation between GHRH values and GH values before injection, significant correlation was observed 6–12 h after estradiol injection. GH and SRIF did not exhibit significant concordance. There was no significant correlation among the neuropeptides (data not shown).

Discussion
Our previous study demonstrated that plasma GH profiles in OVX Shiba goats exhibit distinct pulsatility consisting of conspicuous pulses separated by baseline periods of virtually no GH secretion [5]. It has also been reported that administration of estradiol to OVX goats increased GH pulse amplitude and AUC, whereas pulse frequency and width remained unchanged [5]. Other studies have also shown that plasma GH levels increased after estradiol administration in ruminants [6, 7] and primates [8]. The findings of the present study are strongly consistent with those of previous reports [5–8], supporting a stimulatory effect of exogenous estrogen on GH secretion. Since the GH pulse profiles in the follicular phase are also observed as having a higher amplitude and larger AUC than those in other phases [5], the treatment with estradiol in the present study could be a model for GH pulsatility in the follicular phase of the estrous cycle.

Some studies have reported that estrogen stimulates GH-producing cells. Estrogen activates GH pulsatility via modulation of GH synthesis in the pituitary of rats [36]. Pituitary transcription factor-1, which is involved in the expression of GH mRNA, is upregulated by estrogen in somatotropes of rats [37]. Estradiol supplementa-
tion enhances pituitary sensitivity to GHRH in humans [38]. However, because estrogen receptors are rarely expressed in the pituitary somatotropes in ruminants [39], the main target of estrogen must be cells other than somatotropes, including at least hypothalamic neurons in these species. To evaluate the effects of estradiol on GHRH, SRIF and NPY release in the hypothalamus in detail, we measured fluctuations in the levels in CSF obtained from the third ventricle in conscious goats. Our previous study found a high coincidence of GH pulses with GHRH peaks, no correlation between SRIF and GH and a strong negative correlation between NPY and GH [22]. Before estradiol was injected in the present study, most of GH pulses were also associated with GHRH pulses, and NPY troughs were negatively synchronized with GH pulses, consistent with previous findings. Interestingly, after estradiol was injected, these neuropeptide profiles changed in various fashions.

Neural activities of GHRH are reported to be induced by estrogen in ruminants. With the in vitro perfusion system using bovine anterior pituitary and hypothalamic slices, estradiol increases GHRH secretion but not SRIF secretion [40]. In sheep, the percentage of GHRH neurons expressing c-fos in the hypothalamus is increased by intramuscular injection of estradiol [7, 39]. In this study, after treatment with estradiol, the duration and AUC of the GHRH pulses in the CSF were significantly increased. This finding is not only consistent with previous reports, but also suggests that estradiol increases the amount of GHRH release per pulse. However, the patterns of estrogen receptor expression in GHRH neurons differ among species. Indeed, estrogen receptor (ER) α, but not ER/β, is expressed in GHRH neurons of adult female rats [41]. In contrast, several studies in ewes have reported that GHRH neurons did not express ERα itself, although they were surrounded by ERα [7, 39]. Estradiol thus appears to modulate the activity of GHRH neurons through an indirect pathway in sheep. In goats, ERα was expressed in the arcuate nucleus (ARC) and ventromedial nucleus (VMN) of hypothalamus [42], but whether GHRH neurons express ERα remains unknown. Further studies are needed to determine how estrogen induces GHRH neural activity.

The relationship between estrogen and SRIF neural activity is still unclear. Several studies have reported that estrogen induces expression of SRIF in the VMN of the rat [43] and guinea pig [44]. In sheep, SRIF neurons expressing c-fos in the hypothalamus are increased by estradiol [7, 39]. With the in vitro perfusion system using bovine anterior pituitary and hypothalamic slices, however, estradiol decreases SRIF secretion [40]. Moreover, SRIF neurons of the periventricular nucleus (PeVN), which is the major source of somatostatin in the hypophyseal portal system, do not express ERα in rats or sheep [39, 45]. ERβ expression was not found in that nucleus in rats [45]. The effects of estradiol on SRIF neurons thus appear mainly to be indirect. In this study, after injection of estradiol, SRIF troughs in the CSF tended to be increased and consistent with GH pulses, but they were not significantly changed. According to a report on OVX rats, treatment with both estradiol and progesterone increased SRIF expression, although treatment with estradiol or progesterone alone did not [46]. Taken together, these findings suggest that estrogen alone might not affect SRIF secretion or its contribution to GH pulse generation.

Some investigations have revealed that gonadal steroids modulate the production and release of hypothalamic NPY [47–49]. In particular, estrogen decreases the NPY content and KCl-induced release in rat microdissected hypothalamic sites [49]. In the present study, the baseline of NPY in CSF was drastically decreased after estradiol injection. To our knowledge, this is the first study to find in vivo that estrogen decreases actual NPY release in the hypothalamus. Nevertheless, it remains unknown which NPY neurons were responsible for this. In in vitro experiments in rats, estradiol decreased NPY content selectively in the paraventricular nucleus (PVN), perifornical nucleus, dorsomedial nucleus and lateral hypothalamic area, but not in the VMN [49]. In contrast, in ewes, cell profiles immunoreactive to NPY are found only within the ARC and median eminence [50, 51], and NPY-positive cells in the ARC co-express ERα [51]. In the study of rats, colocalization of NPY and estradiol itself was also determined [52]. Taken together, these findings suggest that some NPY neurons in the hypothalamus are direct targets of estrogen.

The mechanism of generation of GH pulsatility remains controversial. Several patterns of GHRH and SRIF secretion have been reported, which could be associated in a complex fashion with the pulsatile secretion of GH [17, 18]. In our previous study, we determined neuropeptide levels in the CSF of OVX goats and concluded that not only GHRH or SRIF but also NPY might be involved in the generation of GH pulsatility [22]. In this study, after estradiol injection, GHRH pulses were enhanced and baseline NPY release was markedly decreased. Almost all GH secretory events were intrinsically driven by GHRH pulses throughout the pre- and postinjection periods, and GHRH pulses were more strongly associated with GH pulses. In contrast, the correlation between NPY troughs and GH pulses disappeared after estrogen injection. These findings suggest that estrogen regulates the pattern of secretion of GH in the goat by altering secretion of neuropeptides, with enhancement of GHRH pulses and decrease of NPY levels.

It has been reported that NPY could interact with GHRH and SRIF. GHRH and NPY cells exist in the ARC and adjacent areas in rats [53, 54], and NPY fibers overlap GHRH fibers in the external zone of the median eminence in ewes [55]. NPY neurons have also shown to connect SRIF neurons in the PeVN and ARC in rats [56, 57]. Moreover, NPY reportedly stimulates the secretion of both GHRH and SRIF from perfused bovine hypothalamic slices [58], suggesting that NPY could activate these neuropeptide neurons. In this study, however, GHRH, SRIF and NPY had their own intrinsic secretory patterns and did not have significant correlations among them. The further studies are needed to clarify interactions of these neuropeptides in relation to GH pulsatility.

In conclusion, estrogen appears to upregulate the pattern of secretion of GH in goats by altering the secretion of neuropeptides in the hypothalamus. Because GH plays important roles in anaerobic processes governing muscle strength and lipoprotein metabolism [1, 5, 11], the alteration of GH pulsatility observed in this study might affect reproductive fitness in the estrous phase.

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References


receptor (ERα), but not ERβ, gene is expressed in growth hormone-releasing hormone neurons of the male rat hypothalamus. *Endocrinology* 2001; 142: 538–543.


