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Aging alters the circadian rhythm of glucose utilization in the suprachiasmatic nucleus

(middle age/diurnal rhythm/hypothalamus/estradiol/2-deoxyglucose)

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ABSTRACT **We examined the possibility that alterations in the timing of cyclic luteinizing hormone (LH) release during the middle age transition to infertility reflect differences in the circadian pattern of neural function in pacemaker areas of the hypothalamus, particularly the suprachiasmatic nucleus. We measured local cerebral glucose utilization (LCGU) because this parameter is an index of local brain function. We assessed LCGU in several brain areas of young and middle-aged ovariectomized estradiol-treated rats since LH surges are altered when rats are middle-aged. This alteration is correlated with changes in the diurnal pattern of neurotransmitter turnover in several hypothalamic areas that regulate cyclic LH release. The data demonstrate a circadian rhythm in glucose utilization in the dorsal and ventral suprachiasmatic nucleus. In young rats, LCGU increases within 1 hr of lights-on, increases further just prior to the initiation of the LH surge, and decreases within 1 hr of lights-off. In contrast, middle-aged rats show a more gradual increase in LCGU after lights-on, with no further increase prior to the LH surge, and a premature decrease during the afternoon and evening. The data suggest that changes in the circadian pattern of LCGU may be related to the alteration in timing and amplitude of estradiol-induced LH surges in middle-aged rats. Changes in the integrity of the biological clock or in the ability of the biological clock to entrain other neurochemical events may underlie the onset of altered cyclic reproductive function and the transition to irregular estrous cyclicity.**

The female reproductive system provides a unique model system to investigate mechanisms regulating endocrine-dependent aging processes because changes that lead ultimately to infertility and the disappearance of reproductive cycles occur relatively early during adulthood. Thus, one can examine the regulation of aging processes in the virtual absence of age-related pathologies. The reproductive system of the aged female is characterized by the absence of both cyclic gonadotropin release and cyclic ovarian function. The period of total acyclicity and infertility follows a transitional phase during which ovulations occur at increasingly irregular intervals, the probability that a given cycle will be ovulatory is reduced, and the pattern of preovulatory gonadotropin surges and steroid hormone release is altered. Hypothalamic, pituitary, and ovarian functions are altered during the middle-age transitional phase (1–4); however, the relative contribution of each to the transition to reproductive acyclicity is not well understood. The present study tested the hypothesis that a loss in precision of the circadian rhythm of hypothalamic function occurs during middle age, disrupting cyclic reproductive function.

Reproductive cyclicity has a circadian basis and depends on the integrity of a neural pacemaker (5–7). Everett *et al.* (5) proposed that, in rodents, the timing and regularity of preovulatory gonadotropin surges are governed by diurnal neurochemical signals that occur during a "critical period" each day. If these signals are delayed or suppressed sufficiently, preovulatory gonadotropin release is delayed by an entire day and occurs at the proper time 24 hr later. The suprachiasmatic nucleus (SCN) is often called the "biological clock" because its integrity is critical to the maintenance of biological functions that have a circadian basis, including cyclic reproductive function (8–12). Previous studies demonstrated that the diurnal rhythms of norepinephrine and serotonin turnover rates, which accompany cyclic luteinizing hormone (LH) release, are altered in the SCN of middle-aged rats when they enter the transition to age-related estrous acyclicity (13–15). In the present study, we tested the possibility that the reported age-related alterations in the rhythms of neurotransmitter turnover rates reflect a fundamental change in circadian activity in the SCN, possibly contributing to the cascade of events that lead to infertility in senescence. To this end, we measured local rates of glucose utilization in the SCN and other brain areas. Local cerebral glucose utilization (LCGU) is an index of local brain function (16) because the brain uses glucose as its major substrate for energy metabolism. A circadian rhythm in LCGU in the SCN of young male rats (17) has been reported. We studied ovariectomized estradiol-treated young and middle-aged rats because (*i*) diurnal surges of LH occur in young ovariectomized estradiol-treated rats with precise and predictable timing (14, 18, 19), (*ii*) the timing and amplitude of these surges are altered in middle-aged rats entering the transition to reproductive acyclicity (14, 20), and (*iii*) age-related changes in steroid-induced LH surges are associated with changes in the rhythm of turnover of at least some neurotransmitters (13–15). Therefore, we hypothesized that if the timing of several neural events in the SCN were altered with age, middle-aged rats would show a change in the circadian rhythm of glucose utilization in the SCN. We examined LCGU in several other brain areas to determine whether a rhythm is detectable in these brain areas and to assess the effects of age in these brain regions.

MATERIALS AND METHODS

Cycling virgin young (3–4 months old) and middle-aged (12–14 months old) Sprague–Dawley rats (Zivic–Miller, Allison Park, PA) were ovariectomized. Young rats exhibited 4- to 5-day estrous cycles and middle-aged rats exhibited 4- to 7-day estrous cycles prior to ovariectomy. One week later (day 0), they were treated with Silastic capsules containing estradiol (150 μg/ml), such that plasma levels of estradiol were between 20 and 30 pg/ml. The effect of this treatment

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Abbreviations: LH, luteinizing hormone; LCGU, local cerebral glucose utilization; SCN, suprachiasmatic nucleus.

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paradigm on cyclic release of LH and prolactin has been described in detail (14). One day later (day 1), rats were cannulated to the level of the right atrium via the external jugular vein under ether anesthesia. On day 2, LCGU was measured at 0300, 0500, 1000, 1200, 1400, 1700, 1900, and 2300 hr (lights-on between 0400 and 1800 hr) by the autoradiographic 2-deoxy-D-[1-^{14}C]glucose ([^{14}C]deoxyglucose) method (16) with modifications already described (21). In brief, on day 1, rats were cannulated with a Silastic cannula through the jugular vein to the level of the right atrium. At each time that glucose utilization was determined, [^{14}C]deoxyglucose was injected into the right atrium via the indwelling cannula as a bolus at a dose of 75 μCi per kg of body weight (1 Ci = 37 GBq). The cannula was immediately flushed with saline, and timed samples (200 μl each at 0.1, 0.25, 0.50, 0.75, 1.0, 2.0, 3.0, 5.0, 7.5, 10, 15, 25, 35, and 45 min) of mixed venous blood from the right atrium were collected from the same cannula. [^{14}C]Deoxyglucose and glucose concentrations were assessed in blood samples and were used to calculate rates of LCGU. Mixed venous blood samples were drawn with a syringe, and blood volume was replaced with an equal volume of 0.9% saline after each sampling. We have previously shown that the same cannula can be used to administer [^{14}C]deoxyglucose and to collect blood samples without contamination of the originally injected [^{14}C]deoxyglucose (21). This modification reduces the stress of immobilizing the rats in a plaster cast and allowed us to monitor LCGU in unrestrained rats. The procedure was performed under dim red light illumination (40 W) during the dark interval (0300, 1900, and 2300 hr). Immediately before administering [^{14}C]-deoxyglucose, a peripheral blood sample was drawn from the indwelling atrial cannula to assess serum LH concentrations. Twenty-three brain areas were identified by comparing autoradiograms to a stereotaxic atlas (22). They included hypothalamic areas, which regulate gonadotropin release. We focused on the SCN because it is a critical neural pacemaker area of the brain. LCGU was analyzed in the ventral and dorsal aspects of this nucleus separately since previous studies suggested that the neural afferents and efferents to each of these aspects of the SCN, and therefore their functions, may differ (23–25). We assessed LCGU in the medial preoptic nucleus, suprachiasmatic preoptic nucleus, paraventricular nucleus, anterior hypothalamic area, ventromedial nucleus, and pineal gland because rhythms in other indices of neural function occur in these brain areas, and the integrity of these brain areas is critical to circadian functions and/or to the maintenance of cyclic reproductive function (6, 26–29). Other brain areas were assessed because they comprise components of the hypothalamus that are involved in tonic gonadotropin release and/or the maintenance of sexual behavior. Thus, the arcuate nucleus, median eminence, dorsomedial nucleus, and the mammillary bodies (30) are likely sites of interaction between luteinizing hormone-releasing hormone (LHRH) neurons and putative neurotransmitters that regulate LHRH activity. The final group of brain areas are ones that have been previously reported to exhibit age-related alterations in male or ovariectomized female rats (31–33). Thus, the medial and cortical amygdala, motor aspect of the frontoparietal cortex, corpus callosum, nucleus accumbens, striatum, bed nucleus of the stria terminalis, and hippocampus were considered control areas of the brain that are not directly involved in estradiol feedback actions or in reproduction.

Rates of LCGU were calculated from estimates of radioactivity in the selected brain regions and plasma concentrations of [^{14}C]deoxyglucose and glucose, using an operational equation, as described by Sokoloff *et al.* (16). The values for the lumped constant used for the young and middle-aged rats were 0.502 and 0.456, respectively. These values were obtained from linear regression of reported values for 3-, 12-, and 24-month-old male Fischer 344 rats (34) and were applied in the present study based on the assumption that similar changes occur in aging female ovariectomized estradiol-treated Sprague–Dawley rats.

Plasma LH concentrations were measured by a heterologous radioimmunoassay as described (35) with the following modifications: ^{125}I was used to label ovine LH (kindly provided by L. E. Reichert, Albany Medical College). LH-RP2, provided by the National Institute of Diabetes, Digestive and Kidney Diseases Rat Pituitary Hormone Distribution Program, was used as the standard. Anti-ovine LH (GDN15), kindly provided by G. D. Niswender (Colorado State University), was used at a final concentration of 1:400,000. Anti-rabbit immunoglobulin (Antibodies, Inc., Davis, CA) was used at a final concentration of 1:375. All plasma samples were assayed in one assay. The intra-assay coefficient of variation was 6.5%.

Data were analyzed by two-way analysis of variance to determine whether there was an effect of time of day or age. Age-related differences at specific times were analyzed by analysis of simple effects. A limited number of time-related differences were analyzed by Tukey's test (36). To assess whether there was a diurnal rhythm in LCGU within each age group, one-way analysis of variance was performed.

RESULTS

Two days after estradiol treatment, basal plasma LH concentrations at 0300, 0500, 1000, and 1200 hr were similar in young compared to middle-aged ovariectomized rats. In young rats, LH concentrations were increased by 1400 hr, continued to increase at 1700 hr, and returned to baseline levels by 2300 hr (Table 1). In middle-aged rats, LH concentrations were increased at 1700 hr compared to morning values, but concentrations at 1400, 1700, and 1900 hr were significantly lower than in young rats (Table 1). It should be noted that blood samples were not collected at sufficiently frequent intervals to allow completely accurate reflection of profile of the LH surge. However, our previous data (14) establish that the surge of LH in middle-aged rats is markedly attenuated and the onset is delayed by $\approx$1 hr.

A circadian pattern in LCGU was detected in the dorsal and ventral aspects of the SCN of young and middle-aged estradiol-treated ovariectomized rats (Fig. 1). The temporal pattern of this rhythm was dependent on the age of the rat. In young rats, LCGU was low in the morning during the dark (0300 hr) and increased significantly within 1 hr of lights-on. A second increase occurred between 1000 and 1200 hr. LCGU remained high during the afternoon and decreased

Table 1. Plasma LH concentrations (ng/ml; RP-2) in young and middle-aged ovariectomized estradiol-treated rats on day 2

	Time of day, hr							
Age	0300	0500	1000	1200	1400	1700	1900	2300
Young	1.2 ± 0.26	1.7 ± 0.18	1.7 ± 0.33	1.6 ± 0.21	2.6 ± 0.45	13.0 ± 3.91*†	11.3 ± 3.75*†	1.3 ± 0.10
Middle-aged	1.2 ± 0.10	1.7 ± 0.49	1.2 ± 0.09	1.5 ± 0.21	1.8 ± 0.28	2.8 ± 0.59*	1.4 ± 0.21	1.1 ± 0.10

Results are expressed as mean ± SEM (n = 5).
*Significantly greater than LH concentrations at 1200 hr ($P < 0.05$).
†Significantly greater than LH concentrations in middle-aged rats at equivalent time ($P < 0.05$).

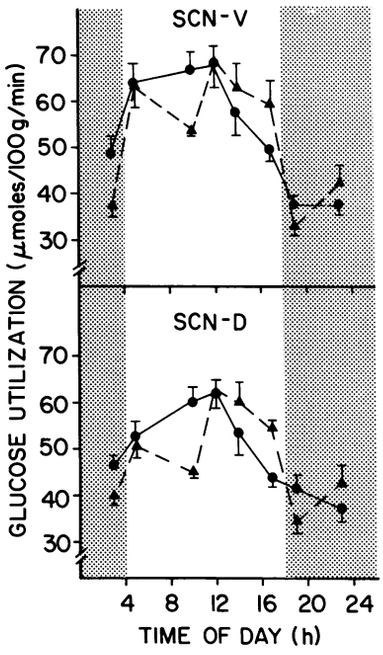

FIG. 1. Glucose utilization in the ventral (SCN-V) and dorsal (SCN-D) aspects of the SCN of young (▲) and middle-aged (●) ovariectomized estradiol-treated rats. In young rats, LCGU increased when lights went on, between 0300 and 0500 hr, and again just before the initiation of the LH surge, between 1000 and 1200 hr. They decreased when lights went off, between 1700 and 1900 hr. In middle-aged rats, LCGU increased between 0300 and 1000 hr and decreased between 1400 and 1900 hr.

significantly within 1 hr of lights-off. Although a circadian rhythm was detected in middle-aged rats in the ventral and dorsal aspects of the SCN, aging altered several distinct aspects of the rhythm (Fig. 1). First, in both the ventral and the dorsal aspects of the SCN, LCGU was low during the dark but was not significantly increased until 1000 hr. Second, in middle-aged rats there was no difference between LCGU at 1000 and 1200 hr. Third, LCGU was higher at 1000 hr in middle-aged compared to young rats. Finally, glucose utilization at 1900 hr, 1 hr after the onset of dark, was lower than at 1400 hr, but not lower than at 1700 hr. LCGU is apparently uniform during the entire light period, with no change at a critical period. Statistical analyses reveal that there was no overall effect of age on LCGU in either the dorsal or the ventral SCN. Thus, only the rhythm of glucose utilization was altered in middle-aged compared to young female rats under ovariectomized estradiol-treated conditions.

A significant effect of time of day was observed in the suprachiasmatic preoptic nucleus and the paraventricular nucleus (Table 2) in young rats. In these brain areas, LCGU was significantly different during the dark (0300, 1900, and 2300 hr) than during the light period. No light/dark rhythm was detectable in middle-aged rats.

We observed an age-related decline in LCGU in the nucleus accumbens and striatum (Table 2). Glucose utilization increased in middle-aged rats in the median eminence (Table 2).

There was a significant interaction between age and time of day in the SCN (Fig. 1), the mammillary bodies, and the pineal gland (Table 2).

DISCUSSION

The general circadian pattern of LCGU that we observed resembles that reported for male rats by Schwartz *et al.* (17). The average LCGU within the SCN, considering all times during the light period (0500, 1000, 1200, 1400, and 1700 hr) was greater than during the dark (0300, 1900, and 2300 hr). A unique aspect of the glucose rhythm in young ovariectomized estradiol-treated rats was the secondary increase during the lights-on period of the day: LCGU increased significantly between 1000 and 1200 hr. This change coincides temporally with an increase in norepinephrine and epinephrine turnover rates in the SCN of equivalently treated rats (13, 37–40). The importance of this change from morning to the occurrence of LH surges during the afternoon is suggested by its absence in other animal models, such as males (17) and ovariectomized females (33), which do not exhibit diurnal surges of LH during the afternoon. In young rats, LCGU was highest at 1200 hr, particularly in the dorsal aspect of the SCN, just prior to LH surge, and remained increased during the time when the LH surge occurred. The data would suggest that elevated neural activity follows a period of relative quiescence (1000 hr), which coincides with the initiation and maintenance of the LH surge.

Although a circadian rhythm in LCGU was detected in middle-aged rats in both the ventral and dorsal aspects of the SCN, several notable differences are apparent. Although there was a significant difference between the rate of glucose utilization during the light vs. dark, the response to lights-on and lights-off was dampened. Glucose utilization increased more slowly after lights went on and the increase did not achieve statistical significance until 1000 hr in both the dorsal and the ventral SCN. LCGU decreased prematurely during the afternoon prior to lights-off and therefore the difference between glucose utilization at 1700 hr (1 hr before lights-off) and 1900 hr (1 hr after lights-off) was not statistically significant in either the dorsal or the ventral SCN. Most important, the secondary increase in LCGU between 1000 and 1200 hr was absent in middle-aged rats. Indeed, LCGU was significantly higher in middle-aged than in young rats at 1000 hr, and there was no difference between morning and afternoon rates of glucose utilization in these older animals. The absence of a change in LCGU between 1000 and 1200 hr correlates with the lack of a diurnal rhythm in norepinephrine turnover rates in the SCN at the same times of day (14). In addition, a diurnal rhythm in serotonin turnover rates was totally absent in middle-aged rats treated with the same hormone protocol (15). Apparently, neural activity is uniform during the entire light period in middle-aged rats, and there is no change in activity at a "critical period" (13, 14). Previous studies suggest that a relative change in neural activity is required at a critical time of day (between 1000 and 1300 hr) to allow LH surges to occur later in the afternoon (13, 14). The present data strongly suggest that such a neural signal is absent in middle-aged rats. It is important to note that there is no overall effect of age on LCGU in the dorsal or ventral SCN; thus, only the rhythm of glucose utilization is altered in middle-aged compared to young rats. A significant age-related decline in LCGU becomes detectable in this brain area only when rats are older (21). Thus, together the data suggest that in this brain area, aging initially affects the rhythm and timing of changes in glucose utilization and later during the aging process affects the absolute rates of glucose utilization. It is interesting to note that despite differences in neural afferents to and efferents from the SCN, the circadian pattern of LCGU and the changes that occur with age were similar in both the dorsal and the ventral aspects of the SCN. This suggests that the rhythmicity of SCN is basic to the entire nucleus and may influence multiple neurotransmitters that traverse the dorsal and ventral aspects of this nucleus.

In young ovariectomized estradiol-treated rats, a significant effect of time of day was observed in two other brain areas, the suprachiasmatic preoptic nucleus and the paraventricular nucleus. Both of these brain areas are important

Table 2. Local cerebral glucose utilization (μmol per 100 g per min) in young and middle-aged ovariectomized estradiol-treated rats

Brain area	Age	Time of day, hr							
		0300	0500	1000	1200	1400	1700	1900	2300
Frontoparietal motor cortex	Young	109 ± 4.6	107 ± 4.9	88 ± 1.1	102 ± 3.0	99 ± 5.6	105 ± 2.2	96 ± 6.3	99 ± 6.4
	Middle-aged	109 ± 9.0	102 ± 3.7	102 ± 3.7	100 ± 3.8	98 ± 4.1	85 ± 4.3	102 ± 4.6	105 ± 5.6
Nucleus accumbens*	Young	68 ± 4.9	65 ± 3.7	66 ± 5.6	69 ± 5.6	67 ± 3.2	74 ± 2.9	64 ± 7.0	62 ± 2.7
	Middle-aged	58 ± 4.3	68 ± 6.6	62 ± 4.1	62 ± 4.4	58 ± 4.3	68 ± 6.6	62 ± 4.1	62 ± 4.4
Striatum*	Young	103 ± 3.6	93 ± 3.5	86 ± 3.1	94 ± 3.8	91 ± 4.5	96 ± 3.4	85 ± 6.1	86 ± 4.8
	Middle-aged	82 ± 5.2	89 ± 6.7	86 ± 2.3	83 ± 4.8	79 ± 1.6	76 ± 4.8	80 ± 5.2	80 ± 2.9
Bed nucleus of stria terminalis	Young	45 ± 3.0	46 ± 3.2	41 ± 1.3	42 ± 1.2	41 ± 2.6	53 ± 8.0	38 ± 2.9	44 ± 1.2
	Middle-aged	43 ± 2.0	43 ± 3.3	45 ± 1.9	43 ± 2.0	42 ± 3.0	35 ± 2.5	38 ± 2.3	38 ± 1.7
Medial preoptic nucleus	Young	40 ± 2.3	48 ± 1.9	46 ± 5.1	42 ± 1.0	44 ± 2.9	44 ± 0.9	36 ± 3.2	44 ± 3.1
	Middle-aged	45 ± 2.2	50 ± 2.8	46 ± 1.9	47 ± 2.0	47 ± 2.5	41 ± 1.6	47 ± 4.9	42 ± 2.7
Suprachiasmatic preoptic nucleus†	Young	39 ± 2.3	47 ± 4.8	43 ± 4.5	45 ± 2.3	40 ± 1.4	41 ± 2.9	33 ± 2.1	38 ± 3.7
	Middle-aged	41 ± 1.0	52 ± 4.9	46 ± 3.2	48 ± 3.4	43 ± 2.0	41 ± 1.5	40 ± 3.9	39 ± 3.2
Paraventricular nucleus†	Young	45 ± 2.0	59 ± 4.6	46 ± 1.6	47 ± 0.8	50 ± 3.5	51 ± 4.2	38 ± 2.1	48 ± 2.1
	Middle-aged	56 ± 5.4	51 ± 2.5	50 ± 3.0	54 ± 3.8	48 ± 4.3	46 ± 2.7	45 ± 1.9	47 ± 4.8
Anteriorhypothalamic nucleus	Young	46 ± 1.8	53 ± 2.6	45 ± 1.9	52 ± 2.8	50 ± 3.6	52 ± 2.9	40 ± 3.8	49 ± 2.6
	Middle-aged	50 ± 2.8	53 ± 2.6	52 ± 2.3	49 ± 2.3	50 ± 4.0	44 ± 1.2	48 ± 3.1	46 ± 3.1
Medial amygdala	Young	42 ± 2.8	47 ± 2.2	37 ± 1.8	45 ± 2.3	41 ± 1.8	43 ± 1.9	35 ± 3.0	45 ± 4.1
	Middle-aged	43 ± 2.1	42 ± 1.9	43 ± 1.5	32 ± 1.7	42 ± 2.0	38 ± 2.7	41 ± 4.5	38 ± 3.1
Cortical amygdala	Young	36 ± 2.1	42 ± 3.1	34 ± 1.0	39 ± 2.2	40 ± 2.2	41 ± 2.4	31 ± 2.9	40 ± 3.8
	Middle-aged	38 ± 1.0	40 ± 2.8	31 ± 7.3	37 ± 1.2	39 ± 2.5	42 ± 5.4	35 ± 3.0	35 ± 2.7
Arcuate nucleus	Young	42 ± 2.1	42 ± 3.2	38 ± 1.6	40 ± 1.2	41 ± 1.6	43 ± 1.8	36 ± 3.3	45 ± 5.1
	Middle-aged	46 ± 1.1	48 ± 2.8	44 ± 2.2	43 ± 3.0	42 ± 1.2	37 ± 1.8	42 ± 2.8	38 ± 2.8
Median eminence*	Young	58 ± 5.2	59 ± 2.0	43 ± 1.6	45 ± 2.8	45 ± 3.8	54 ± 4.0	48 ± 5.8	56 ± 5.5
	Middle-aged	59 ± 4.1	74 ± 5.8	62 ± 4.9	62 ± 2.5	66 ± 5.8	59 ± 11.0	57 ± 3.0	51 ± 6.2
Dorsomedial nucleus	Young	51 ± 1.6	53 ± 3.0	52 ± 3.5	52 ± 2.0	50 ± 3.0	57 ± 3.3	45 ± 3.4	51 ± 4.3
	Middle-aged	53 ± 3.1	59 ± 3.6	53 ± 2.3	55 ± 4.1	52 ± 3.0	50 ± 2.3	52 ± 3.6	46 ± 2.9
Ventromedial nucleus	Young	40 ± 1.8	46 ± 3.0	45 ± 2.9	42 ± 2.6	42 ± 2.5	44 ± 1.5	37 ± 3.5	43 ± 4.0
	Middle-aged	44 ± 2.7	47 ± 2.6	43 ± 1.5	42 ± 2.7	40 ± 1.6	42 ± 3.0	42 ± 3.8	43 ± 9.0
Hippocampus	Young	63 ± 3.5	65 ± 2.7	54 ± 3.4	59 ± 2.3	59 ± 3.7	67 ± 3.2	52 ± 3.2	61 ± 4.6
	Middle-aged	58 ± 1.5	61 ± 4.8	62 ± 1.2	58 ± 3.0	58 ± 4.7	52 ± 1.8	59 ± 4.2	52 ± 6.0
Medial mammillary body‡	Young	122 ± 7.2	124 ± 2.7	110 ± 3.8	108 ± 3.4	108 ± 2.6	121 ± 4.3	93 ± 3.2	126 ± 8.5
	Middle-aged	127 ± 1.8	116 ± 4.8	109 ± 4.6	110 ± 3.0	108 ± 5.3	99 ± 1.0	122 ± 8.2	109 ± 6.7
Pineal‡	Young	63 ± 3.5	72 ± 4.0	62 ± 3.1	63 ± 2.5	73 ± 8.8	77 ± 2.8	55 ± 5.1	82 ± 9.4
	Middle-aged	69 ± 8.8	60 ± 4.3	76 ± 4.9	69 ± 3.6	73 ± 11.0	74 ± 4.2	90 ± 8.4	79 ± 6.2

Results are expressed as mean ± SEM (n = 4 or 5).
*Significant effect of age ($P < 0.05$).
†Significant effect of time of day in young rats only ($P < 0.05$).
‡Significant interaction between age and time of day ($P < 0.05$).

in the maintenance of cyclic reproductive function. Efferent connections leave the suprachiasmatic preoptic nucleus and project to other hypothalamic nuclei, including the paraventricular nucleus. Lesions of the paraventricular nucleus abolish diurnal rhythms in pineal *N*-acetyltransferase activity, but do not interfere with the circadian rhythm of locomotor activity (41). Lesions of the suprachiasmatic preoptic nucleus lead to a state of constant vaginal estrous, which is characterized by the lack of cyclic gonadotropin release and the absence of ovulation (42). It is noteworthy that no significant LCGU rhythm could be detected in either the paraventricular nucleus or the suprachiasmatic preoptic nucleus of middle-aged rats. It is possible that the lack of a diurnal rhythm in LCGU in the paraventricular and suprachiasmatic preoptic nucleus of middle-aged rats may also contribute to alterations in cyclic reproductive function.

Other brain areas, such as the ventromedial nucleus (29) and cerebral cortex (43), have been reported to exhibit diurnal rhythmicity in multiple neurochemical parameters. However, we did not detect a diurnal rhythm in LCGU in these brain areas with ovariectomized estradiol-treated rats in the present study. The lack of a rhythm in LCGU in these brain areas may be because (*i*) the resolution of the deoxyglucose technique is not adequate to detect a rhythm, (*ii*) multiple neural rhythms with counterbalancing patterns exist in these brain areas, such that no overall rhythm can be detected, or (*iii*) the particular neurochemical index that exhibits a circadian rhythm does not involve the majority of cells in this brain area.

In summary, the data demonstrate that the SCN exhibits a circadian rhythm in LCGU that is altered when female rats reach middle age, a period of transition to infertility. The data suggest that aging involves a change in the integrity of the biological clock. This may involve a loss in the entrainment of the SCN to the photoperiod or a change in the ability of the SCN to modulate the rhythmic activity of multiple neurotransmitters.

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