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Research article

Age-dependent changes in 24-hour rhythms of catecholamine content and turnover in hypothalamus, corpus striatum and pituitary gland of rats injected with Freund's adjuvant

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Abstract

Background: Little information is available on the circadian sequela of an immune challenge in the brain of aged rats. To assess them, we studied 24-hour rhythms in hypothalamic and striatal norepinephrine (NE) content, hypothalamic and striatal dopamine (DA) turnover and hypophysial NE and DA content, in young (2 months) and aged (18–20 months) rats killed at 6 different time intervals, on day 18th after Freund's adjuvant or adjuvant's vehicle administration.

Results: Aging decreased anterior and medial hypothalamic NE content, medial and posterior hypothalamic DA turnover, and striatal NE concentration and DA turnover. Aging also decreased NE and DA content in pituitary neurointermediate lobe and augmented DA content in the anterior pituitary lobe. Immunization by Freund's adjuvant injection caused: (i) reduction of DA turnover in anterior hypothalamus and corpus striatum; (ii) acrophase delay of medial hypothalamic DA turnover in old rats, and of striatal NE content in young rats; (iii) abolition of 24-h rhythm in NE and DA content of neurointermediate pituitary lobe, and in DA content of anterior lobe, of old rats.

Conclusions: The decline in catecholamine neurotransmission with aging could contribute to the decrease of gonadotropin and increase of prolactin release reported in similar groups of rats. Some circadian responses to immunization, e.g. suppression of 24-h rhythms of neurointermediate lobe NE and DA and of anterior lobe DA were seen only in aged rats.

Background

In 1974, Pittendrigh and Daan first described the changes caused by aging in the circadian timing system of rodents [1]. Since then, a bulk of information has accumulated indicating that reduced amplitude, shorter free-running periods and desynchronization of circadian rhythms are associated with advanced age in rodents as well as in humans (for references see [2]). Both the efficacy of input and output pathways from the central nervous system circadian pacemaker, located in the

hypothalamic suprachiasmatic nuclei, and the functioning of the central pacemaker itself, change with advancing age. In addition, some of the decline in overt circadian rhythmicity may be due to deteriorating function of the body's aging effector systems [2].

An aspect of circadian organization in aged subjects less known is the modification in amplitude or phase of circadian rhythms during the different phases of the response to an immune challenge. Since aging is associated with declines in multiple areas of immune function [3], it seems feasible that differences in the circadian response to an immune challenge with age occur.

Adjuvant arthritis is an experimental model for rheumatoid arthritis, induced by the intradermal injection of heat-killed Mycobacterium tuberculosis in incomplete Freund's adjuvant to rats [4]. In the classical adjuvant-induced arthritis model, polyarthritis is accompanied by a widespread systemic disease. In this experimental model we previously reported the effect of aging on circadian organization of plasma prolactin, growth hormone (GH), thyrotropin (TSH), insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone, studied during the acute phase of inflammatory disease of the joints (18 days after Freund's adjuvant injection) in rats [5]. As a continuation of those studies we now report, in comparable groups of animals, the changes in 24-hour organization of hypothalamic and striatal norepinephrine (NE) content and dopamine (DA) turnover and hypophysial NE and DA content. The results support the view that 24-h rhythms and levels of hypothalamic, striatal and hypophysial catecholamines are age-dependent, as are some of the responses to Freund's adjuvant administration.

Results

Figure 1 shows the 24-h changes in hypothalamic NE content in young and old rats injected with Freund's adjuvant or its vehicle 18 days earlier. Rhythm parameters as analyzed by Cosinor are summarized in Table 1. In the three hypothalamic regions examined significant 24-h changes of NE content occurred, as shown by individual oneway analysis of variance (ANOVA) (Fig. 1). In the experimental groups in which data fitted a cosine function, acrophases coincided with the second half of activity span or the first half of rest span (04:06 – 13:08 h. Table 1). A factorial ANOVA taking age as a main factor revealed a significantly lower NE content in anterior and medial hypothalamus of aged rats ($F_{1,126}$= 8.76, $p < 0.004$, and $F_{1,122} = 4.83$, $p < 0.03$, respectively). In Cosinor, mesor values of NE content in anterior hypothalamus and amplitude values in posterior hypothalamus of old rats were significantly lower than their respective younger counterparts (Table 1).

Figure 2 depicts the 24-h changes in hypothalamic DA turnover. Individual one-way ANOVA's indicated significant time-of-day changes in the anterior hypothalamus of all experimental groups, in the medial hypothalamus of young rats treated with Freund's adjuvant and of old rats treated with adjuvant's vehicle, and in the posterior hypothalamus of young and old rats treated with adjuvant's vehicle. Acrophases occurred during the second half of activity span or first half of rest span (04:00 – 13:47 h), except for the medial hypothalamus of Freund's adjuvant-treated young rats whose acrophases were at the beginning of the activity span (20:17 h. Table 1). A factorial ANOVA taking age as a main factor indicated that medial and posterior hypothalamic DA turnover was significantly lower in old rats ($F_{1,124} = 5.29$, $p < 0.01$ and $F_{1,121} = 6.41$, $p < 0.001$, respectively). Mean values (pg/mg) of 3,4-dihydroxyphenylacetic acid (DOPAC) and DA in young rats were: 38 ± 2.7 and 147 ± 11 (anterior hypothalamus); 83 ± 5.5 and 72 ± 4 (medial hypothalamus); 10 ± 1 and 27 ± 2.3 (posterior hypothalamus). Mean values (pg/mg) of DOPAC and DA in old rats were: 31 ± 2 and 122 ± 9 (anterior hypothalamus); 65 ± 6.6 and 68 ± 5 (medial hypothalamus); 7 ± 1 and 26 ± 1.7 (posterior hypothalamus). In every hypothalamic region of old rats DOPAC concentration was lower than that of young rats ($p < 0.05$, Student's t test). When immunization was taken as a main factor in factorial ANOVA, a significant reduction in DA turnover was found in the anterior hypothalamus of Freund's adjuvant-treated rats ($F_{1,123}$ = 3.99, $p < 0.04$). Mean values (pg/mg) of DOPAC and DA in the anterior hypothalamus of adjuvant's vehicle- and Freund's adjuvant-treated rats were: 37 ± 2.3 and 125 ± 13, and 33 ± 1.9 and 130 ± 12, respectively.

Figure 3 depicts striatal NE content and DA turnover. Striatal NE content varied significantly on a 24-h basis. Acrophases coincided with the second half of activity span or the first half of rest span (06:29 – 12:16 h), except for young rats treated with Freund's adjuvant in which acrophase occurred late in the rest span (17:15 h) (Table 2). Differences in acrophases between immunized and non-immunized young rats were significant (Table 2). Analyzed as a main factor in a factorial ANOVA, old rats had lower striatal NE concentrations ($F_{1,121} = 8.76$, $p < 0.003$). In every case, old rats showed smaller mesor values of striatal NE content than their younger counterparts (Table 2). Amplitude decreased significantly in old rats injected with Freund's adjuvant (Table 2).

Time-of-day changes in striatal DA turnover were not significant as indicated by individual one-way ANOVA's (Fig. 3). In a factorial ANOVA, the effects of both main factors "age" and "immunization" were significant, aging and Freund's adjuvant decreasing striatal DA turnover ($F_{1,122} = 5.97$, $p < 0.01$ and $F_{1,123} = 13.4$, $p < 0.00001$, re-

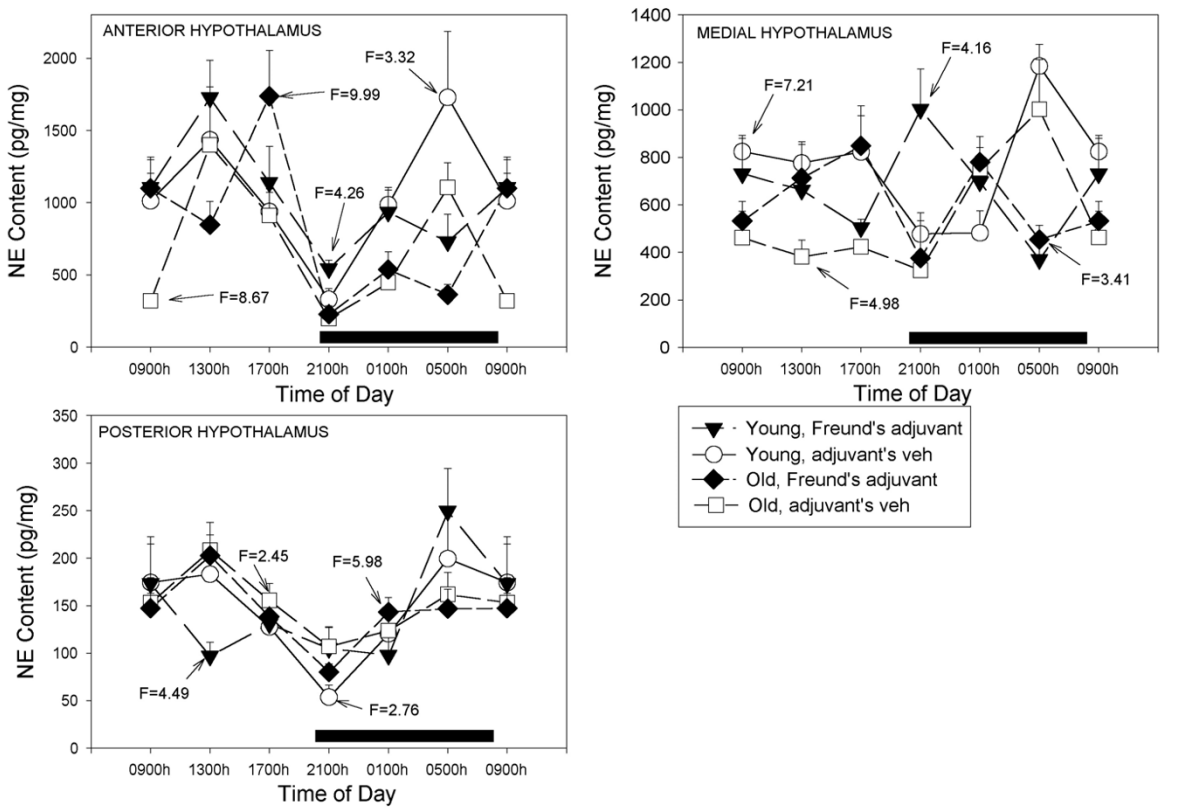

Figure 1
Twenty-four h changes of hypothalamic NE content in young and old rats injected with Freund's adjuvant or its vehicle 18 days earlier. Groups of 5–7 rats were killed by decapitation at six different time intervals throughout a 24-h cycle. Shown are the means + SEM. The groups in which significant differences among time intervals were detected by a one-way ANOVA are indicated by their F values in the Figure. For further statistical analysis, see text.

spectively). Mean values (pg/mg) of striatal DOPAC and DA in young and old rats were: 437 ± 52 and 3123 ± 225, and 189 ± 21 and 2631 ± 202, respectively. Differences in striatal DOPAC concentration between young and old rats were significant ($p < 0.01$, Student's t test). Mean values (pg/mg) of striatal DOPAC and DA in adjuvant's vehicle-and Freund's adjuvant-treated rats were: 411 ± 54 and 2417 ± 256, and 215 ± 25 and 2152 ± 234, respectively. The decrease in striatal DOPAC concentration after Freund's adjuvant administration was significant ($p < 0.01$, Student's t test).

Hypophysial NE and DA levels are depicted in Fig. 4. NE levels of the neurointermediate lobe showed significant 24-h variations in all groups except for old rats receiving Freund's adjuvant. Acrophases were at 02:31 – 03:34 h (Table 3). In a factorial ANOVA in which age was analyzed as a main factor, a significant depression of NE content was detected in aged rats ($F_{1,123} = 10.1$, $p < 0.0001$). Mesor values were significantly lower in old rats. Amplitude in old rats receiving adjuvant's vehicle was significantly lower than that of their respective young counterparts (Table 3).

Significant 24-h changes in DA content of the neurointermediate lobe occurred in the 4 experimental groups, except for old rats receiving Freund's adjuvant (Fig. 4); acrophases were at 02:47 – 04:23 h (Table 3). A factorial ANOVA indicated a depressive effect of aging on neurointermediate lobe DA content ($F_{1,126} = 28.4$, $p < 0.00001$). In Cosinor, mesor and amplitude values in old rats receiving adjuvant's vehicle were significantly lower than those of their respective young counterparts (Table 3).

Table 1: Cosinor analysis on 24-h changes in hypothalamic NE content and DA turnover in young and old rats injected with Freund's adjuvant or its vehicle 18 days earlier.

	Mesor	Amplitude	Acrophase (h, min)	Percent of rhythm
NE CONTENT				
Anterior hypothalamus				
Young, Freund's adjuvant	1030 ± 99	428 ± 34	12:36 ± 02:01	65 ± 12
Young, adjuvant's vehicle	1073 ± 113	444 ± 56	08:09 ± 02:35	51 ± 6.5
Old, Freund's adjuvant	803 ± 77*	499 ± 66	13:08 ± 02:13	48 ± 6.6
Old, adjuvant's vehicle	731 ± 98*	-	-	-
Medial hypothalamus				
Young, Freund's adjuvant	661 ± 76	-	-	-
Young, adjuvant's vehicle	762 ± 55	225 ± 35	08:40 ± 02:08	44 ± 5.9
Old, Freund's adjuvant	618 ± 64	-	-	-
Old, adjuvant's vehicle	557 ± 43	285 ± 43	04:06 ± 02:34	70 ± 6.9
Posterior hypothalamus				
Young, Freund's adjuvant	142 ± 32	55 ± 9	06:24 ± 02:44	49 ± 9.7
Young, adjuvant's vehicle	143 ± 13	62 ± 11	08:50 ± 02:03	81 ± 10.1
Old, Freund's adjuvant	143 ± 33	36 ± 4*	10:35 ± 01:33	53 ± 7.8
Old, adjuvant's vehicle	152 ± 27	38 ± 6*	11:26 ± 02:22	70 ± 9.2
DA TURNOVER				
Anterior hypothalamus				
Young, Freund's adjuvant	0.26 ± 0.01	-	-	-
Young, adjuvant's vehicle	0.27 ± 0.03	0.08 ± 0.01	06:51 ± 01:02	64 ± 3.4
Old, Freund's adjuvant	0.24 ± 0.02	0.05 ± 0.02	13:47 ± 01:47	59 ± 6.4
Old, adjuvant's vehicle	0.30 ± 0.01	0.05 ± 0.01	09:28 ± 02:21	53 ± 7.7
Medial hypothalamus				
Young, Freund's adjuvant	1.04 ± 0.01	0.22 ± 0.03	20:17 ± 02:33	62 ± 7.7
Young, adjuvant's vehicle	1.16 ± 0.02	n.s.	n.s.	n.s.
Old, Freund's adjuvant	0.96 ± 0.02	n.s.	n.s.	n.s.
Old, adjuvant's vehicle	0.96 ± 0.03	-	-	-
Posterior hypothalamus				
Young, Freund's adjuvant	0.34 ± 0.01	n.s.	n.s.	n.s.
Young, adjuvant's vehicle	0.38 ± 0.02	0.05 ± 0.02	09:20 ± 02:36	29 ± 4.4
Old, Freund's adjuvant	0.32 ± 0.02	n.s.	n.s.	n.s.
Old, adjuvant's vehicle	0.30 ± 0.01	0.07 ± 0.01	04:00 ± 01:35	39 ± 4.9

Shown are the means ± S.E.M. (n = 5–7/group). Mesor and amplitude values are expressed as pg/mg (NE content) or as DOPAC/DA ratios. Percent of rhythm defines the part of variation that could be explained by a cosine function in Cosinor. Asterisks designate significant differences ($p < 0.05$) as compared to the respective young counterparts in a one-tailed Student's t test. n.s.: not significant daily changes in a oneway ANOVA; (-): not significant changes in Cosinor

Figure 4, lower panel, depicts DA levels in the anterior hypophysis. Significant 24-h changes were observed in all groups except for old rats administered with Freund's adjuvant. Only changes in young animals injected with adjuvant's vehicle fit a cosine function, with acrophase at 10:31 h. In a factorial ANOVA, aging augmented anterior hypophysial DA levels ($F_{1,122} = 37.8$, $p < 0.00001$).

Discussion

The present study, performed in rats sacrificed at 6 different time intervals during a 24-h cycle, documented the following effects of aging on hypothalamic, striatal and hypophysial catecholamines: (i) lower NE content in anterior and medial hypothalamus; (ii) lower DA turnover in medial and posterior hypothalamus; (iii) lower striatal NE concentration and DA turnover; (iv) lower NE and DA content of the neurointermediate pituitary lobe; (v) augmented anterior hypophysial DA content. In addition, the identified effects of Freund's adjuvant administration were: (i) reduction of DA turnover in the anterior hypothalamus; (ii) acrophase delay of DA turnover in medial hypothalamus of old rats; (iii) acrophase delay of striatal NE content of young rats; (iv) decreased striatal DA turnover; (v) abolished 24-h variations of neurointermediate lobe NE and DA content, and of anterior lobe DA content, in old rats.

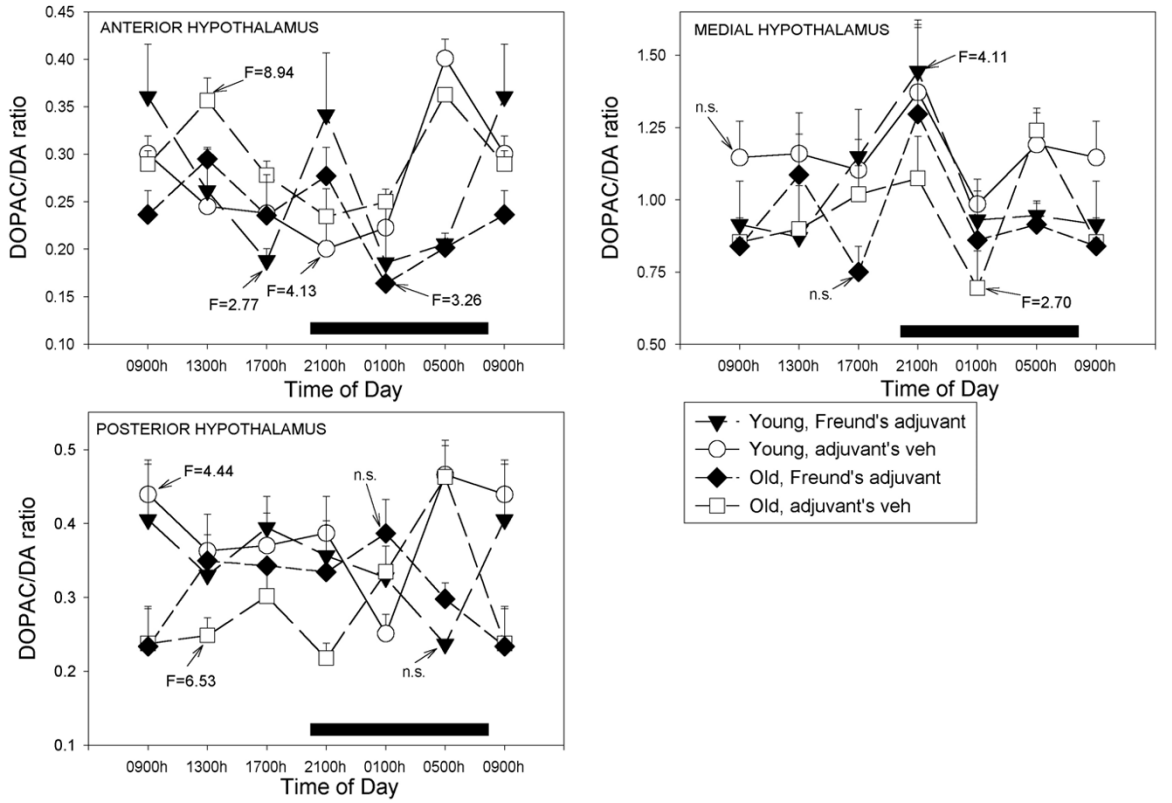

Figure 2
Twenty-four h changes of hypothalamic DA turnover in young and old rats injected with Freund's adjuvant or its vehicle 18 days earlier. Groups of 5–7 rats were killed by decapitation at six different time intervals throughout a 24-h cycle. Shown are the means + SEM. The groups in which significant differences among time intervals were detected by a one-way ANOVA are indicated by their F values in the Figure (n.s.: not significant F). For further statistical analysis, see text.

Most previous studies on the effect of aging on brain and peripheral catecholamines in rats have been obtained as single time points in a 24-h cycle. Decreases in NE content in limbic areas, spinal cord, medulla oblongata and pons, and less consistently, in the hypothalamus and corpus striatum of old rats were identified [6–12]. In aging male rats, levels of hypothalamic NE either decreased [6,13–16] or remained unchanged [9,17,18].

Our present results indicate that aged rats had lower NE content in the anterior and medial hypothalamus. In addition, hypothalamic NE levels showed significant 24-h changes with acrophases at the second half of activity span or first half of rest span. In the posterior hypothalamus of old rats, amplitude of rhythm was significantly lower than in their respective younger counterparts. Since noradrenergic neurons stimulate LH releasing hormone release [19], the decline in noradrenergic stimulation with aging could contribute to the age-related decrease of FSH and LH described previously in similar groups of rats [5]. It should be noted that a strong positive correlation did exist between the rate constant of NE loss measured in rats and the magnitude of the age-related depletion in NE concentrations within specific brain regions [20].

The present results also support the existence of a lower DA turnover in medial and posterior hypothalamus of aged rats. This was the consequence of a decrease of both DA and DOPAC concentration, being most marked in the case of the latter. DA released by tuberoinfundibular dopaminergic neurons in the hypothalamic periventricular and arcuate nuclei tonically inhibits pituitary prolactin secretion. In turn, prolactin stimulates DA secretion

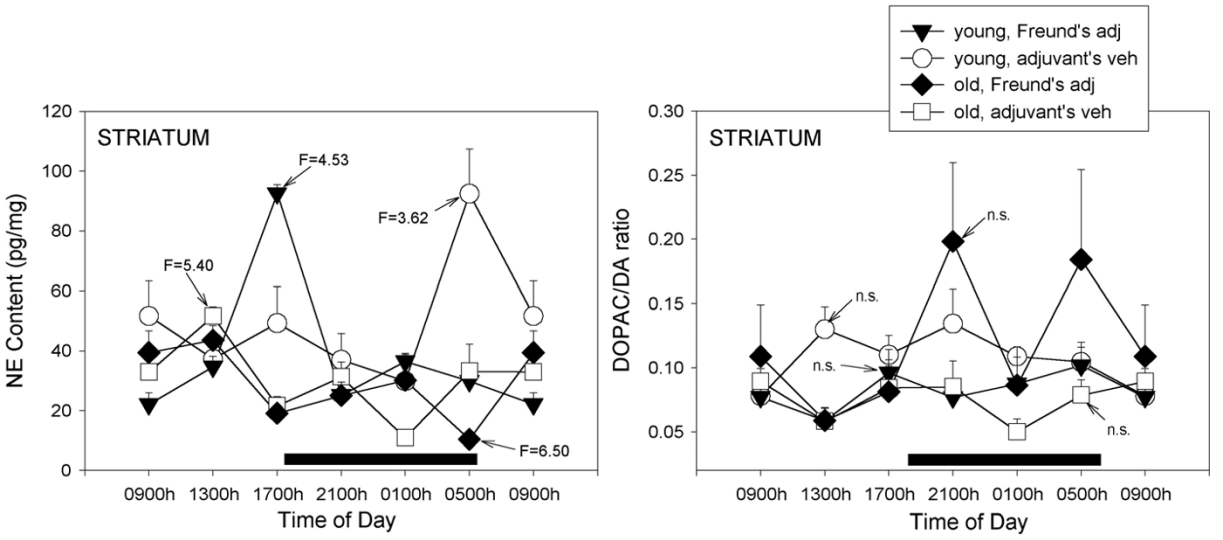

Figure 3
Twenty-four h changes of NE content and DA turnover in corpus striatum of young and old rats injected with Freund's adjuvant or its vehicle 18 days earlier. Groups of 5–7 rats were killed by decapitation at six different time intervals throughout a 24-h cycle. Shown are the means + SEM. The groups in which significant differences among time intervals were detected by a one-way ANOVA are indicated by their F values in the Figure (n.s.: not significant F). For further statistical analysis, see text.

from dopaminergic neurons, forming a feedback loop [21,22]. This autoregulatory feedback control of prolactin is altered in the aged rat, as evidenced by increased circulating concentrations of prolactin and decreased activity of these neurons [22,23]. In addition, tuberoinfundibular DA neurons of old rats failed to respond to exogenous prolactin administration [24].

Prolactin increases occur with aging in male rats of most strains, including Wistar [25] and Long-Evans [26] rats, but not in all, e.g. Sprague-Dawley [27] rats. Most studies have reported a decrease in DA content in the hypothalamus and median eminence of aging male rats, including Wistar [14,15], Long-Evans [28], Sprague-Dawley [9] and F344 [16] rats. In the present study, medial hypothalamic, as well as median eminence DA levels (data not shown), decreased with age. Since the decreases in DOPAC levels exceeded those of DA, DA turnover rate also decreased. Our results agree with measurements of hypothalamic DA turnover in aged Wistar [14] and Long-Evans [28] rats at single time-points in a 24-h cycle.

In vivo, secretion of DA into hypophysial portal blood has been reported to decrease [29] or to increase with aging [30]. Long-term (12 months) testosterone replacement in 24-month-old male Wistar rats increased DA release in the medial preoptic area nearly to levels observed in 3-month-old rats [31]. These findings suggest that DA is involved in the age-related decline in male reproductive function, as indicated by the low FSH, LH and testosterone levels reported in our previous study [5].

In rats, the concentrations of DA in the adenohypophysis increases progressively with age. The increase in the DA content is not a consequence of reduced metabolism of DA, nor is it a static pool because as in young rats, adenohypophysial DA is rapidly decreased by pharmacological treatments that reduce the activity of tuberoinfundibular DA neurons. In aged rats, the amount of DA associated with light particles was 5 times that found in young rats, whereas the amount of DA associated with heavy particles was the same as that in young rats [32]. Therefore, an increased concentration of DA in anterior hypophysis, like that reported in the present study in old rats, does not necessarily result in inhibition of prolactin secretion. It is interesting that, in addition to DA, impairments are observed in the processing (binding, accumulation and intracellular distribution) of hypothalamic hormones in the adenohypophysis of old rats. Taken together, these observations are supportive of the view that the neuroendocrine/endocrine changes appearing with age result from a complex balance of functional alterations occurring at each level, central and peripheral, of the axis.

In the present study, striatal DA and DOPAC levels and turnover rate decreased significantly in aged rats. The re-

Table 2: Cosinor analysis on 24-h changes in striatal NE content and DA turnover in young and old rats injected with Freund's adjuvant or its vehicle 18 days earlier.

	Mesor	Amplitude	Acrophase (h, min)	Percent of rhythm
NE content				
Young, Freund's adjuvant	40.1 ± 5.2	21.1 ± 3.6	17:15 ± 01:34	39 ± 4.5
Young, adjuvant's vehicle	49.6 ± 6.6	16.8 ± 2.1	06:29 ± 01:02#	33 ± 5.6
Old, Freund's adjuvant	27.9 ± 4.1*	8.5 ± 2.8**	12:16 ± 02:01	27 ± 5.0
Old, adjuvant's vehicle	30.3 ± 5.4*	12.5 ± 2.2	11:49 ± 02:21	50 ± 7.6
DA turnover				
Young, Freund's adjuvant	0.11± 0.03	n.s.	n.s.	n.s.
Young, adjuvant's vehicle	0.19 ± 0.03*	n.s.	n.s.	n.s.
Old, Freund's adjuvant	0.04 ± 0.03	n.s.	n.s.	n.s.
Old, adjuvant's vehicle	0.15 ± 0.05#	n.s.	n.s.	n.s.

Shown are the means ± S.E.M. (n = 5–7/group). Mesor and amplitude values are expressed as pg/mg (NE content) or as DOPAC/DA ratio. Percent of rhythm defines the part of variation that could be explained by a cosine function in Cosinor. Asterisks designate significant differences (*$p < 0.05$; **$p < 0.01$) as compared to their respective young counterparts; #$p < 0.05$ as compared to their respective Freund's adjuvant-treated counterparts (one-tailed Student's t test), n.s.: not significant daily changes in a one-way ANOVA.

sults agree with the bulk of information obtained in aged rodents indicating decreased levels and turnover rate of DA [8,9,16,28,33–36]. Generally, the decreased levels of midbrain DA and DOPAC detectable in aged rats have little correlation with age-related changes in the density of dopaminergic receptor binding or the density of DA uptake sites [37].

Significant amounts of NE and DA are present in the neurointermediate lobe of the hypophysis. DA nerve terminals belong to centrally located neurons whereas the origin of NE fibers is in part peripheral, as shown by the 40–50% decrease of posterior pituitary NE content after the bilateral removal of the sympathetic superior cervical ganglia [38,39]. Activation of central noradrenergic input to the magnocellular nuclei augments arginine vasopressin release [40] whereas that of peripheral noradrenergic input decreased arginine vasopressin release [39]. Data presented herein indicate that reduced concentrations of DA occurred in the neurointermediate lobe of the pituitary gland of old rats as compared to those of young rats. Our present results also indicate that NE levels in neurointermediate lobe showed significant 24-h variations in all groups except for old rats receiving Freund's adjuvant. Acrophases were at about the middle of the activity span (at 02:47 – 04:23 h), in concordance with the previously reported maxima in tyrosine hydroxylase activity in the superior cervical, stellate and celiac-superior mesenteric ganglia of young and old rats [41]. Therefore, the central and peripheral sources of noradrenergic innervation of hypophysial neurointermediate lobe seem to have similar 24-h patterns of activation. NE content in neurointermediate lobe and tyrosine hydroxylase in sympathetic ganglia [41] was lowest in old rats.

A number of studies have indicated an age-related reduction in immune function associated with cell mediated immunity in both experimental animals and humans. In advancing age alterations were mainly observed in T cell mediated immunity including decreased proliferative responsiveness of T cells to mitogens, decreased T cell-dependent humoral immune responses, lowered resistance to tumor cell challenge, decreased graft-vs.-host reactivity, delayed skin allograft rejection time, impaired delayed hypersensitivity, reduced cytolytic immune response, altered cytokine production after stimulation, and decreased natural killer cell activity (for references see [42]). Most of the studies were performed at single time points in a 24-h cycle, thus overseeing any effect the circadian system can have on the responses.

In the present study, the injection of Freund's adjuvant to young and old rats was employed as an antigenic challenge. The adjuvant-induced arthritis that follows is considered to be a model for T-lymphocyte-dependent, autoimmune diseases [43] and the central symptoms found partly constitute the "sickness behavior", i.e., the behavioral changes that accompany the immune reaction [44]. One feature of sickness behavior addressed experimentally in the present study was the modification of the 24-hour pattern of hypothalamic, striatal and hypophysial catecholamines occurring 18 days after the injection of complete Freund's adjuvant to young and old rats.

Changes in circadian rhythms are apparent at an early phase of experimental arthritis in rats and persist there-

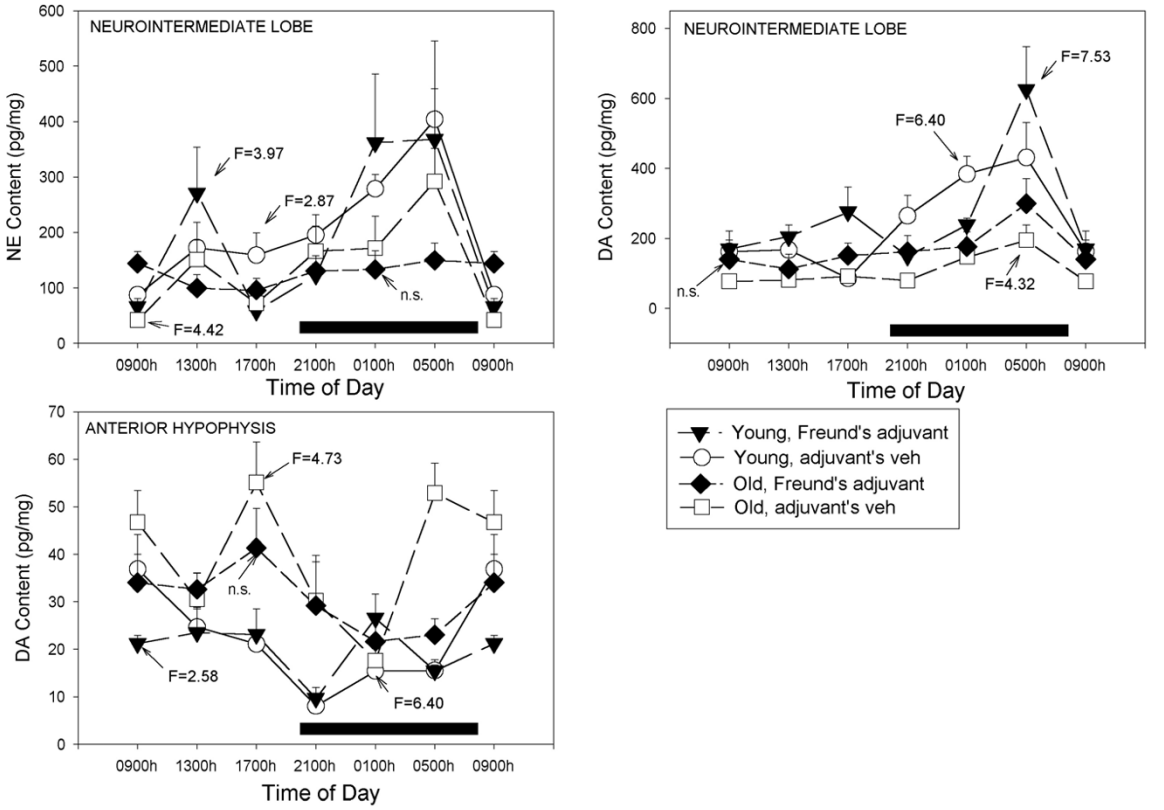

Figure 4
Twenty-four h changes of NE and DA content in hypophysial neurointermediate lobe and of DA content of hypophysial anterior lobe in young and old rats injected with Freund's adjuvant or its vehicle 18 days earlier. Groups of 5–7 rats were killed by decapitation at six different time intervals throughout a 24-h cycle. Shown are the means + SEM. The groups in which significant differences among time intervals were detected by a one-way ANOVA are indicated by their F values in the Figure (n.s.: not significant F). For further statistical analysis, see text.

after [45–49]. In the present study Freund's adjuvant administration brought about, 18 days later, a decrease of DA turnover in the anterior hypothalamus and striatum. It also caused a number of circadian alterations, including acrophase delay of DA turnover in medial hypothalamus of old rats, acrophase delay of striatal NE content of young rats and abolished 24-h variations of neurointermediate lobe NE and DA content, and anterior lobe DA content, in old rats. Experimental evidence suggests that symptomatology after Freund's adjuvant administration are a part of a defense response to antigenic challenge and are mediated by the neural effects of cytokines like interleukin (IL)-l, IL-6, IL-2, granulocyte-macrophage colony-stimulating factor and interferon-α [44,50]. Since we previously reported that immunosuppression restored rhythmicity of several of the neuroendocrine parameters examined in Freund's adjuvant-injected rats [51], the immune-related nature of the studied phenomena seems to be warranted. Suprachiasmatic nuclei themselves may be sensitive to immune-derived signals. Presumably, the chronic stress condition given by mycobacterial adjuvant injection is instrumental in inhibiting a number of circadian rhythms at early and late phases of disease.

Conclusions

Aged rats had lower NE content in the anterior and medial hypothalamus, smaller amplitude of 24-h rhythm in posterior hypothalamic NE content, lower DA turnover in medial and posterior hypothalamus, augmented adenohypophysial DA and decreased NE and DA content in pituitary neurointermediate lobe. These results are prob-

Table 3: Cosinor analysis on 24-h changes in hypophysial NE and DA content, in young and old rats injected with Freund's adjuvant or its vehicle 18 days earlier.

	Mesor	Amplitude	Acrophase (h, min)	Percent of rhythm
NE CONTENT				
Neurointermediate lobe				
Young, Freund's adjuvant	208 ± 32	117	03:34 ± 01:09	40 ± 7.1
Young, adjuvant's vehicle	216 ± 26	102 ± 14	02:31 ± 02:02	51 ± 6.5
Old, Freund's adjuvant	125 ± 15*	n.s.	n.s.	n.s.
Old, adjuvant's vehicle	149 ± 19*	69 ± 8*	02:34 ± 00:56	37 ± 4.4
DA CONTENT				
Neurointermediate lobe				
Young, Freund's adjuvant	277 ± 43	125 ± 23	04:23 ± 02:00	30 ± 6.2
Young, adjuvant's vehicle	250 ± 40	171± 48	02:47 ± 01:54	86 ± 12
Old, Freund's adjuvant	174 ± 20*	n.s.	n.s.	n.s.
Old, adjuvant's vehicle	112 ± 42*	49 ± 22*	03:25 ± 01:54	61 ± 11
Anterior hypophysis				
Young, Freund's adjuvant	20 ± 3	-	-	-
Young, adjuvant's vehicle	21± 4	11± 3	10:31 ± 01:05	75 ± 11
Old, Freund's adjuvant	30 ± 6*	n.s.	n.s.	n.s.
Old, adjuvant's vehicle	39 ± 7*	-	-	-

Shown are the means ± S.E.M. (n = 5–7/group). Mesor and amplitude values are expressed as pg/mg. Percent of rhythm defines the part of variation that could be explained by a cosine function in Cosinor. Asterisks designate significant differences ($p < 0.05$) as compared to the respective young counterparts in a one-tailed Student's t test. n.s.: not significant daily changes in a one-way ANOVA; (-): not significant changes in Cosinor

ably instrumental for the neuroendocrine/endocrine changes appearing with age. Aged rats had also significantly decreased striatal DA and DOPAC levels and turnover rate when measured at 6 different time points along a 24-h cycle, thus agreeing with the bulk of information obtained at single time points on the occurrence of age-related decreased levels and turnover rate of DA in corpus striatum.

With advancing age alterations were mainly reported on T cell mediated immunity. We hereby reported that during Freund's adjuvant-induced arthritis (a T-lymphocyte-dependent autoimmune disease) a decrease of DA turnover occurred in the anterior hypothalamus and striatum. We also observed that responses to immunization like suppression of 24-h rhythms of neurointermediate lobe NE and DA and of anterior lobe DA were only seen in aged rats. Collectively, our results indicate that 24-h rhythms and levels of hypothalamic, striatal and hypophysial catecholamines are age-dependent, as are some of the responses to Freund's adjuvant administration.

Materials and Methods

Animals

Experiments were carried out in adult male Wistar rats, kept under light between 0800 and 2000 h daily. Light intensity at the level of the animal cages was about 200 lux. Rats had access to food and water ad libitum. Adequate measures were taken to minimize pain or discomfort, in accordance with the principles and procedures outlined in European Communities Council Directives (86/609/EEC).

Groups of young (2 months) and aged (18–20 months) rats were injected s.c. with Freund's complete adjuvant (0.5 mg heat-killed Mycobacterium butyricum/rat) or its vehicle (0.5 ml paraffin oil containing 15% mannide monooleate) 3 h after light on (HALO) (i.e., at 11:00 h). The effect of varying the time of Freund's adjuvant injection on day-night differences of submaxillary lymph node ornithine decarboxylase activity (an index of lymph node proliferative response) was examined in a previous work [52]. Immunization performed during daylight (5 HALO) or at night (18 HALO) resulted in similar day-night differences in ornithine decarboxylase activity, indicating that changes in lymph node proliferative responses were relatively independent of the biological time of mycobacterial antigen exposure [52]. We maintained a similar injection schedule as in several previous studies conducted on Freund's adjuvant effects on immune and endocrine responses [53,54]; thus, rats included in the present study were injected with Freund's adjuvant at 3 HALO (11:00 h).

Although arthritis is induced most easily in inbred Lewis rats, it is also produced, to a milder extent, in Wistar rats [55–58]. Rats injected with Freund's adjuvant vehicle were included as a control of any inflammatory reaction the adjuvant's oil alone might cause [59–61]. The course of adjuvant-induced arthritis was followed by behavioral observations including those of spontaneous behavior-mobility, exploring, rearing and scratching [4,43]. Eighteen days after Freund's adjuvant injection a lack of mobility and exploring behavior, an increase in scratching behavior and signs of hyperalgesia were clearly established in young and old rats as compared with their respective adjuvant's vehicle-injected groups. As reported previously by using plethysmography [62], old rats exhibited less behavioral signs of inflammation (spontaneous behavior-mobility, exploring, scratching) than young rats.

On day 18 after injection, groups of 5–7 rats were killed by decapitation at 6 different time intervals throughout a 24-hour cycle. The brains were quickly removed and the hypothalamus, corpus striatum and pituitaries were taken out. The hypothalamus was further sectioned in the frontal plane, the anterior and posterior regions comprising one-third of the block each [63].

Catecholamine assays

Tissue was weighed and homogenized in chilled (0–1°C) 2 M acetic acid. After centrifugation (at 15 000 × g for 30 min, at 5°C), the samples were analyzed by high performance liquid chromatography using electrochemical detection (Coulochem, 5100A, ESA; USA). AC-18 reverse phase column eluted with a mobile phase (pH 4, 0.1 M sodium acetate, 0.1 M citric acid, 0.7 mM sodium octylsulphate and 0.57 mM EDTA containing 10% methanol, v/v), was employed. Flow rate was 1 ml/min, at a pressure of 2200 psi. Fixed potentials against H_2/H^+ reference electrode were: conditioning electrode: -0.4 V; preoxidation electrode: +0.10 V; working electrode: +0.35 V. Catecholamine concentrations were calculated from the chromatographic peak heights by using external standards. The linearity of the detector response for NE, DA and DOPAC (a major catabolite of DA) was tested within the concentration ranges found in hypothalamic supernatants. The turnover of DA was assessed by the DOPAC/DA ratio [64]. Although DOPAC concentration depends on the balance between catabolite synthesis and clearance, its acidic nature slows substantially its clearance from tissue, so that DOPAC concentration reflects an integral of past DA release.

Statistical analysis

Statistical analysis of results was performed by a one-way ANOVA, a two-way factorial ANOVA or one-tailed Student's t test, as stated. Cosinor analysis was used to analyze general rhythmic parameters, i.e., acrophase (the maximum of the cosine function fit to the experimental data), mesor (the statistical estimate of the 24-h time series mean) and amplitude (half the difference between maximal and minimal values of the derived cosine curve). Percent of rhythm defined the part of variation that could be explained by a cosine function. Statistical analysis of Cosinor parameters was carried out by standard procedures [65]. Statistical significance of the derived cosine curves was tested against the null hypothesis (i.e., amplitude = 0) [66]; p values lower than 0.05 were considered evidence for statistical significance.

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