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Recovery of Diminished Mealtime-Associated Anticipatory Behavior by Aniracetam in Aged Rats

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TANAKA, Y., M. KURASAWA AND K. NAKAMURA. *Recovery of diminished mealtime-associated anticipatory behavior by aniracetam in aged rats.* PHARMACOL BIOCHEM BEHAV **66**(4) 827–833, 2000.—Disease- or age-related neuropsychiatric symptoms and cognitive and chronobiological impairments greatly aggravate the activities of daily living (ADL) in patients. The present study evaluates the effects of aniracetam on a decline in mealtime-associated anticipatory behavior in aged rats, as an animal model of temporally regulated behaviors or habitual daily activities. Aged rats showed a lower but typical nocturnal motor activity rhythm than young rats when the animals were fed ad lib. Mealtime-associated anticipatory behavior emerged in young rats when the rats were fed at a fixed time for 6 days, but the activity in aged rats was diminished. Repeated administration of aniracetam (100 mg/kg PO) or physostigmine (0.1 mg/kg SC) for 7 days ameliorated the impaired anticipatory behavior in aged rats. Nefiracetam (10 mg/kg PO) was ineffective. All compounds tested had no effect on appetite or motor ability. These results indicate that aging disturbs the timing or temporal regulation of anticipatory behavior, probably resulting from dysfunction in a food-entrainable oscillator linked to central cholinergic systems. The restoration of the time-keeping ability by aniracetam may be mediated by the facilitation of reticulothalamic cholinergic neurotransmission, and the action may lead to the improvement of declined ADL in stroke patients. © 2000 Elsevier Science Inc.

Mealtime-associated anticipation Temporal regulation of behavior Cholinergic system Aniracetam Aged rats Activities of daily living

ELDERLY patients with cerebrovascular or Alzheimer's disease are also afflicted with neuropsychiatric symptoms, cognitive deficits, and chronobiological impairments (13,23,35). Those symptoms retard physical and cognitive rehabilitation therapies, and thereby aggravate activities of daily living (ADL) and decrease quality of life (7). Among them, lack of spontaneity and motivation, delirium and depression, as well as dementia, are pivotal factors that interfere with the therapy (9,28,29). Moreover, disease- or age-related disturbance of circadian rhythmicity may be a fundamental part of such mental states (13,35).

Many studies have suggested a close association between central cholinergic deficits and cognitive and behavioral abnormalities in animals and humans (1,24,26). Concerning chronobiological systems, cholinergic modulation is believed to be important in the temporal regulation of behaviors and memory of time (8,21). Indeed, vascular dementia of multi-infarction or Binswanger type is characterized by reduced levels of cholinergic markers, such as acetylcholine (ACh), choline acetyltransferase activity, and muscarinic ACh receptors in the brain (4,27,34), and the presence of circadian rhythm disorders, sleep disorders, and behavioral problems (10,13, 23,24). Therefore, cholinergic drugs may be helpful in treating functional loss or disability due to cerebral infarction or hemorrhage, by increasing cognitive ability and by improving neuropsychological disruptions, such as emotional liability and behavioral problems.

Aniracetam, a cognition enhancer, is clinically used to treat emotional disturbances, sleep disorders, and behavioral abnormalities in stroke patients (10,23), and in patients with Parkinson's disease (6). In animals, aniracetam has been demonstrated to ameliorate experimentally induced learning and memory impairments (12), delirium (18,19), and also abnormal behaviors (5,33). Although the action mechanisms of

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aniracetam are not yet fully clarified, central cholinergic activation (i.e., enhanced ACh release and increased choline acetyltransferase activity) has so far been proposed as the main mechanism (12,18,20).

Recent clinical studies indicate that aniracetam accelerates rehabilitation therapy, and consequently, improves ADL (personal communication). However, few or no studies have been reported that experimentally evaluated the effects of aniracetam on age- or disease-related deterioration in temporal regulation of behaviors or ADL, because of the lack of an adequate animal model. Therefore, the present study was designed to support the clinical usefulness of aniracetam by investigating its effects on the temporal regulation of behaviors. For this purpose, we used mealtime-associated anticipatory behavior that was attenuated or diminished in aged rats (14,30).

METHOD

Animals

Male Wistar rats were obtained from Charles River Japan and housed in groups of about three rats per cage. They were kept in a room with a controlled temperature of 22 ± 2°C and relative humidity of 55 ± 10%, with lights on from 0730 to 1930 h. The animals had free access to water and food (CRF-1, Charles River Japan) until the start of experiment. The study was carefully performed according to the "Principles of Laboratory Animal Care," which were dictated by the Animal Care and Use Committee at Nippon Roche Research Center and approved by the Japanese authorities.

Mealtime-Associated Motor Activity

Young (9 weeks old) and aged (around 30 months old) rats were used. The experiment was performed by the method of Shibata et al. (30), with a minor modification. Briefly, each rat was individually housed in a plastic cage (21 × 32 × 26 cm) to become habituated to the novel circumstances for 1 week before the experiment. Motor activity was measured with the AB system (Neuroscience Inc, Tokyo, Japan), which was designed to detect the infrared radiation from rats, and the activity counts were recorded at 1-h intervals. After the rats were fasted for 24 h (day 0), they were fed ad lib for only 1 h from 1330 h on 6 consecutive days (days 1 to 6). On day 7, food was withheld again. Animals had free access to water throughout the experiment. The average of daily food intake was measured before (days −6 to −2) and during (days 2 to 6) the restriction periods. Test compounds were given to rats immediately after the termination of the feeding time, once daily for 7 consecutive days from days 1 to 7. The mealtime-associated anticipatory motor activity (% counts) was defined as the proportion of activity counts during a 2-h period from 1130 h to the total activity counts for 24 h. Light and dark periods commenced at 0730 and 1930 h, respectively.

Drugs

The compounds used here were aniracetam (Ro13-5057) and nefiracetam (Ro64-0673), both of which were synthesized at F. Hoffmann–La Roche (Basle, Switzerland), and physostigmine hemisulfate (Sigma, St. Louis, MO). Both aniracetam and nefiracetam suspended in 0.25% carboxymethyl cellulose (CMC) solution containing 1–2 drops of Tween 80 were given orally in a volume of 5 ml/kg, and physostigmine dissolved in 0.9% saline was given subcutaneously in a volume of 1 ml/kg. Control rats were given 0.25% CMC solution as a vehicle under the same administration schedule.

Data Analysis

Differences between groups were statistically analyzed with one-way analysis of variance (ANOVA) for repeated-measures designs followed by multiple comparison of the Ryan-Einot-Gabriel-Welsch method for repeatedly measured data, or with Student's *t*-tests and one-way ANOVA followed by Dunnett's *t*-tests. The percentage data were modified by angular transformation for one-way ANOVA. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Difference in Circadian Motor Activity and Anticipatory Behavior Between Young and Aged Rats

When we compared the difference in circadian motor activity rhythm between young and aged rats, each group exhibited a typical nocturnal rhythm of motor activity before food restriction (day −1) (Fig. 1). However, the nocturnal and to-

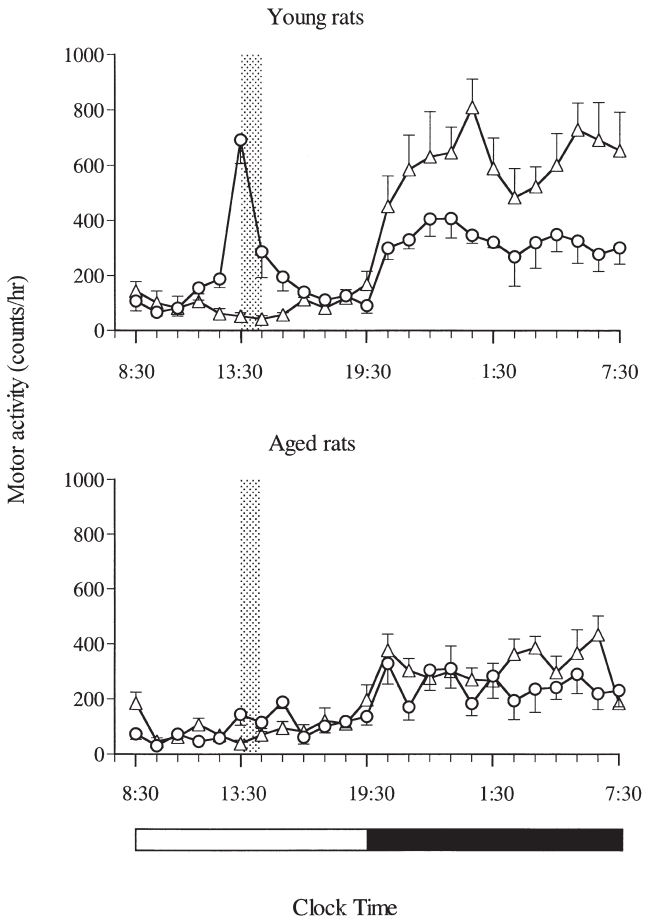

FIG. 1. Circadian locomotor activity rhythm and mealtime-associated anticipatory behavior in young and aged rats. After rats were fasted for 24 h (day 0), feeding was restricted to only 1 h from 1330 h for 6 consecutive days (days 1 to 6). Food was withheld again on day 7. Data show means ± SEM of locomotor activity measured every 1 h, obtained from seven rats/group. Dotted column; daily feeding period, △ day −1, ○ day 7.

tal daily activities in aged rats were significantly ($p < 0.01$ and $p < 0.05$, respectively) lower than those in young rats, and thereby, the L/D ratio in aged rats was much higher ($p < 0.001$) (Table 1). During the food-restriction periods (days 6 and 7), there was a significant, $F(6, 2) = 17.7$, $p < 0.001$, increase in the diurnal motor activity in young rats in comparison with that on day −1, whereas little or no increase was observed in aged rats. The increase seen in young rats reflected the development of mealtime-associated anticipatory behavior and appetitive behavior itself. In contrast, the nocturnal motor activity was significantly, $F(6, 2) = 18.1$, $p < 0.001$, decreased only in young rats (Fig. 1 and Table 1). Consequently, the total daily activity in young rats was significantly, $F(6, 2) = 10.9$, $p < 0.01$, reduced, whereas the L/D ratio was elevated in both groups, $F(6, 2) = 28.1$, $p < 0.001$, in young rats, and $F(5, 2) = 8.64$, $p < 0.05$ or $p < 0.01$, in aged rats (Table 1). The food anticipatory motor activity for 2 h prior to the feeding time emerged remarkably, $F(6, 2) = 159$, $p < 0.001$, in young rats on days 6 and 7, and it was greatly higher on day 7 (+992%) than on day 6 (+358%). In contrast, the increase in aged rats was insignificant compared with the motor activity on day −1. Respective average food intake per 100 g of body weight before (ad lib feeding for 24 h) and during (only for 1 h per day) the food-restriction period for aged rats was significantly ($p < 0.001$) smaller than those for young rats because of the heavier body weights in aged rats, but food intake during the food restriction period was similarly reduced to about 40% of ad lib feeding in both groups (Fig. 2).

Effects of Aniracetam and Other Compounds on Diminished Anticipatory Behavior in Aged Rats

Next, we examined the effects of aniracetam on the mealtime-associated anticipatory behavior found to be diminished on days 6 and 7 in aged rats. Repeated administration of aniracetam at daily oral doses of 30 and 100 mg/kg had no effects on either diurnal and nocturnal motor activities on days 6 and 7 (Table 2) or the excitatory motor activity induced by the drug treatment (data not shown), compared with those of the vehicle control. The treatment also had no effect on gross behaviors. Aniracetam, however, improved the aging-dependent impairment of the emergence of mealtime-associated anticipatory activity on day 7, but not on day 6, in a dose-dependent manner, and significantly attenuated it at 100 mg/kg, $F(2, 13) = 4.18$, $p < 0.05$ (Table 2 and Fig. 3). Physostigmine, at a daily subcutaneous dose of 0.1 mg/kg, also restored the impaired anticipatory activity, $F(2, 13) = 3.88$, $p < 0.05$, on day 7, with a significant, $F(5, 2) = 10.3$, $p < 0.01$, increase in the diurnal motor activity (Table 3). The compound, however, induced mild salivation and hypomotility in most of the animals. Nefiracetam, at a daily oral dose of 10 mg/kg, caused a nonsignificant tendency toward development of mealtime-associated anticipatory activity (Table 3). Daily food intake during the food-restricted periods was unaffected by any of the tested compounds (Fig. 2).

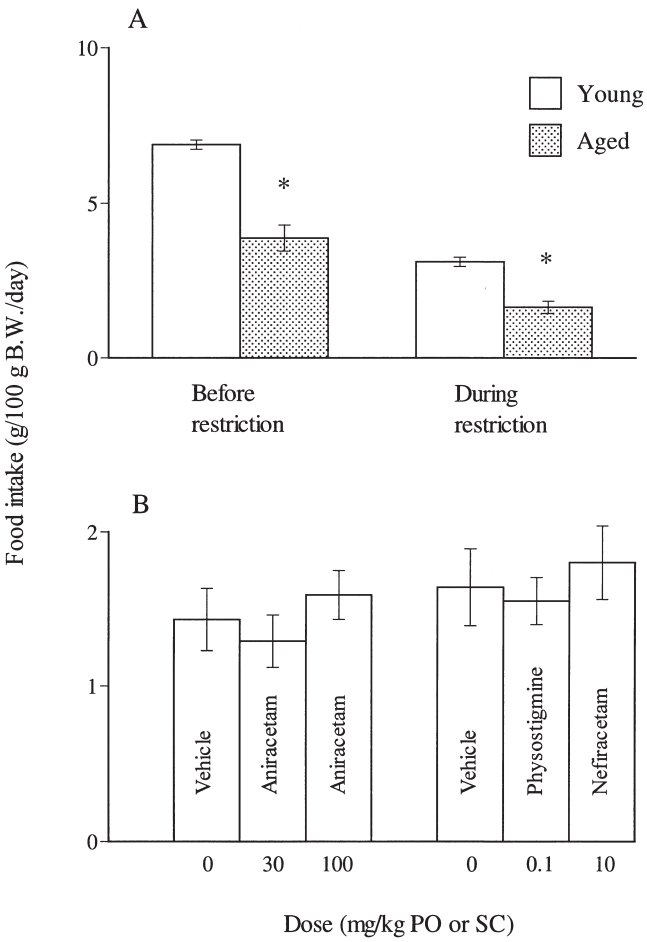

FIG. 2. Daily food intake before and during food restriction of young and aged rats (A), and drug effects on food intake during the food-restricted period in aged rats (B). Average food intake (g/100 g body weight/day) was measured before (days −6 to −2) and during (days 2 to 6) food restriction. Data show means ± SEM obtained from four to nine rats/group. *$p < 0.001$; a significant difference from the young group (Student's *t*-test).

TABLE 1

COMPARISON OF DIURNAL AND NOCTURNAL MOTOR ACTIVITY AND MEALTIME-ASSOCIATED ANTICIPATORY BEHAVIOR BETWEEN YOUNG AND AGED RATS

Group	Period	Motor Activity (Counts) Day −1	Day 6	Day 7
Young	Light	1123 ± 219	2431 ± 475¶	2236 ± 366¶
	Dark	7397 ± 822	3950 ± 272¶	3953 ± 348¶
	Total	8520 ± 997	6382 ± 624#	6189 ± 607#
	L/D ratio	0.15 ± 0.02	0.62 ± 0.10¶	0.57 ± 0.07¶
	Anticipation (1130–1330)	1.30 ± 0.27	5.96 ± 0.66¶	14.2 ± 1.10¶
Aged	Light	1177 ± 181	1400 ± 177§	1140 ± 156*
	Dark	3819 ± 438†	3222 ± 597	2990 ± 562
	Total	4995 ± 605*	4620 ± 745	4129 ± 687*
	L/D ratio	0.30 ± 0.02‡	0.48 ± 0.05#	0.41 ± 0.04§
	Anticipation (1130–1330)	2.23 ± 0.25*	3.38 ± 0.43†	4.61 ± 0.91‡

Diurnal and nocturnal motor activities were measured during the light (730 to 1930) and dark (1930 to 730) periods. The total daily activity corresponded to a sum of both activities. Anticipatory behavior was defined as % counts. Data show means ± SEM obtained from six to seven rats/group.

*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$; significant differences from the young group (Student's *t*-test). §$p < 0.05$, #$p < 0.01$, ¶$p < 0.001$; significant differences from corresponding day −1 values (repeated measures one-way ANOVA followed by the Ryan-Einot-Gabriel-Welsch test).

TABLE 2

EFFECTS OF ANIRACETAM ON DAILY MOTOR ACTIVITY AND MEALTIME-ASSOCIATED ANTICIPATORY BEHAVIOR IN AGED RATS

Treatment	Dose (mg/kg)	Period	Motor Activity (Counts) Day −1	Day 6	Day 7
Vehicle	—	Light	1174 ± 195	1119 ± 110	859 ± 102
		Dark	5271 ± 1271	2068 ± 384†	2008 ± 353†
		Total	6445 ± 1413	3188 ± 446†	2866 ± 370†
		L/D ratio	0.26 ± 0.03	0.51 ± 0.05§	0.39 ± 0.06‡
		Anticipation (1130–1330)	2.53 ± 0.37	3.99 ± 0.52	4.19 ± 1.10
Aniracetam	30 PO	Light	1243 ± 295	1260 ± 163	928 ± 146
		Dark	5179 ± 1143	2058 ± 174‡	2168 ± 177‡
		Total	6422 ± 1345	3319 ± 277†	3096 ± 202†
		L/D ratio	0.27 ± 0.07	0.63 ± 0.08§	0.45 ± 0.08‡
		Anticipation (1130–1330)	2.36 ± 0.73	3.74 ± 0.50	5.26 ± 1.05†
	100 PO	Light	825 ± 176	1139 ± 92.5	960 ± 70.1
		Dark	2967 ± 275	1879 ± 128§	1882 ± 156§
		Total	3793 ± 439	3018 ± 183§	2841 ± 192§
		L/D ratio	0.29 ± 0.04	0.61 ± 0.05§	0.52 ± 0.05§
		Anticipation (1130–1330)	1.27 ± 0.38	3.31 ± 1.01	8.23 ± 0.99*

After rats were fasted for 24 h (day 0), feeding was restricted to only 1 h from 1330 for 6 consecutive days (days 1 to 6). Food was withheld again on day 7. Aniracetam was administered once daily for 7 consecutive days (days 1 to 7). Anticipatory behavior was defined as % counts. Data show means ± SEM obtained from four to six rats/group.

*$p < 0.05$; a significant difference from vehicle (one-way ANOVA followed by Dunnett's t-test). †$p < 0.05$, ‡$p < 0.01$, §$p < 0.001$; significant differences from day −1 (repeated measures one-way ANOVA followed by the Ryan-Einot-Gabriel-Welsch test).

DISCUSSION

In the present study, we confirmed that mealtime-associated anticipatory behavior, which was measured as an index of the circadian time-keeping or temporal regulation of behavior was diminished with aging, consistent with the previous reports (14,30). Moreover, we demonstrated that the repeated administration of aniracetam restored the impairment of temporal regulation of behavior, as did physostigmine.

Food restriction lowered the nocturnal activity in young rats to the level of the aged rats, while aged rats exhibited similar nocturnal activity under both nonrestricted and restricted conditions. Thus, there may be an adaptive behavioral strategy to minimize energy consumption to offset food restriction especially in young rats. These results indicate that food-entrainable oscillator influences the light-entrainable oscillator, as revealed by the hypothesis that both oscillator systems are weakly coupled (32). Moreover, it is suggested that the light-entrainable oscillator is functionally attenuated with aging, and the interaction with the food-entrainable oscillator may also be suppressed, consistent with the previous report (36).

The mealtime-associated anticipatory behavior in aged rats was diminished but not reduced. In fact, the motor activity for 2 h prior to the feeding time did not significantly change among days −1, 6, and 7. In addition, vehicle-treated aged rats showed no appearance of anticipatory activity on day 7, as seen in Fig. 3. Moreover, aged rats exhibited much less behavioral adaptation to food restriction than did the young rats. Although it is uncertain whether the behavioral adaptation correlates with anticipatory behavior positively or negatively, the present results in young rats seem to suggest rather the positive link. Therefore, the adaptation deficit in aged rats might diminish the anticipatory behavior. Thus, although the amount of anticipatory activity depends on the measure of activity, the present results are thought to clearly indicate an age-related change in the timing or temporal regulation of behavior but not in the magnitude of anticipatory activity. Consequently, anticipatory behavior seems to be impaired by aging (14,30).

It is unlikely, however, that the general hypoactivity in aged rats inhibited an emergence of mealtime-associated anticipatory behavior. Haloperidol evoked hypoactivity in rats but it did not prevent the emergence of the food-entrained rhythm (15). MK-801 inversely induced hyperlocomotion in rats and impaired food-anticipatory activity rhythm (22). The food consummatory motivation in aged rats appeared to be normal, as revealed by the fact that the rate of food intake reduction was approximately the same in both groups when ad lib feeding was switched to restricted feeding.

It has been proposed that central cholinergic systems are involved in the temporal regulation of behavior (21). Recently, there have been successive reports of a neurochemical correlation between central cholinergic neuronal activity and anticipation. For example, extracellular concentrations of ACh in the rat hippocampus and frontal (prefrontal) cortex were increased during the anticipation phase preceding meal presentation (3,8). The ACh release in the frontal cortex could be selectively enhanced by the animal's past training

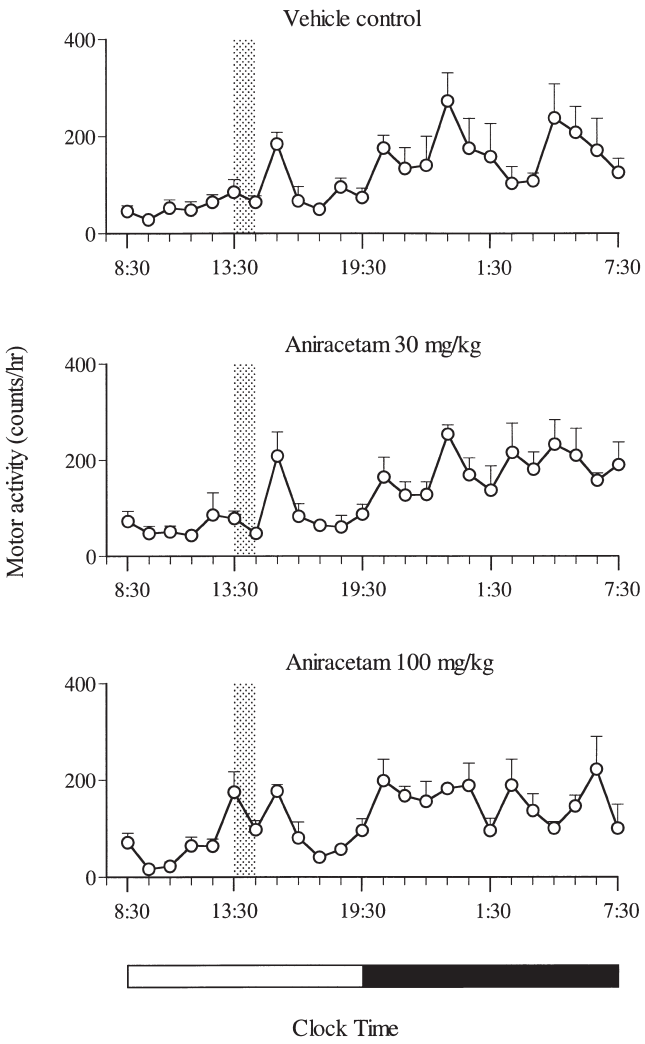

FIG. 3. Effects of aniracetam on circadian motor and mealtime-associated anticipatory activity rhythms on day 7 in aged rats. Aniracetam was given orally to rats immediately after the termination of feeding time once daily for 7 consecutive days (days 1 to 7). Data show means ± SEM obtained from five to six rats/group.

experience, perhaps from the anticipation of the predicted reward (8), while the diurnal release was changed with aging (16). Thus, the temporal regulatory mechanism of anticipatory behavior appears to be clearly linked to ACh function in the frontal cortex (8). In contrast, in the studies identifying the localization of candidate substrates for the food-entrainable oscillator, the neocortex and limbic structures, such as hippocampus, amygdala, nucleus accumbens, and medial forebrain anterior to the thalamus, appeared not to be implicated in the food-anticipatory circadian rhythm in rats (15), and neither was the suprachiasmatic nucleus (2). It has also been reported that aging impairs the food- or mealtime-entrainable circadian oscillator and time-keeping systems (14,30). Taken together with the present findings, it is most likely that the deterioration of chronobiological systems, which generate and entrain circadian anticipatory rhythm, in aged rats may be partially due to age-related cholinergic dysfunction in caudal diencephalic and brainstem structures (11,15,25). Attenuation by cholinergic drugs of impaired mealtime-associated anticipatory activity rhythm in old rats (21) strongly supports that suggestion.

The restoration by either aniracetam or physostigmine of diminished mealtime-associated anticipatory behavior in aged rats on day 7 was not accompanied by an alteration in the circadian spontaneous motor activity rhythm, diurnal and nocturnal activities, and food consumption. These results suggest that the recovery of anticipatory behavior elicited by both compounds is not related to an increased appetite motivation or locomotor activation. Moreover, aniracetam failed to alter premature response (an index of anticipation) in the two-lever choice reaction task of scopolamine-treated middle-aged rats, in which the weight is usually maintained at 80% of their free-feeding weight and the animals can acquire a food pellet as a positive reinforcer when they chose and press the correct lever (18). Those observations presumably indicate that aniracetam may affect temporal regulation of anticipatory behavior rather than anticipatory ability/capacity.

We recently demonstrated that the repeated oral administration of aniracetam preferentially activated the reticulothalamic cholinergic pathway in stroke-prone spontaneously hypertensive rats (SHRSP) with cholinergic deficits (20). The activation of the cholinergic pathway and of the subsequent thalamocortical noncholinergic pathway by aniracetam not only ameliorates scopolamine-induced attention deficits and low vigilance (18), but also may improve age-related functional loss of the time-keeping system and reentrain the circadian anticipatory behavior. In practice, local perfusion of *N*-anisoyl-γ-aminobutyric acid (*N*-anisoyl-GABA), a major metabolite of aniracetam, but not aniracetam itself, into the pedunculopontine tegmental nucleus (Ch5) or nucleus reticularis thalami enhanced ACh release in the nucleus reticularis thalami of freely moving SHRSP (20), probably via group II metabotropic glutamate receptors as evidenced in the prefrontal cortex of SHRSP (31). Thus, aniracetam may restore the time-keeping systems, through a facilitation of the reticulothalamic cholinergic neurotransmission (12,18,20), by stimulating the food-entrainable circadian oscillator and/or by enhancing the temporal regulation of circadian behavior.

In contrast, nefiracetam, one of the 2-pyrrolidinone derivatives like aniracetam, elicited no significant recovery of mealtime-associated anticipatory behavior, despite its reported cholinergic effect (17). The discrepancy between both pyrrolidinones may be explained by a difference in the target cholinergic pathway, its underlying mechanism or effect on GABAergic function. In particular, the facilitation of GABAergic neurotransmission by nefiracetam may inhibit ACh release associated with food anticipation in the rat brain (3,17).

Functional loss of the time-keeping system would appear as sleep disorders, circadian rhythm disorders, nocturnal behavioral problems, and decreased ADL in the elderly and in patients with cerebrovascular or Alzheimer's diseases. Therefore, age-related disturbance of the mealtime-associated anticipatory behavior may be a useful animal model of circadian rhythmicity or ADL deficiency. Indeed, aniracetam is effective in the restoration of decreased ADL and spontaneity, as well as reduced wakefulness and concentration among the sequelae of cerebral infarction (23), and in Parkinson's disease (6). Furthermore, aniracetam concomitantly administered with zopiclone was also efficacious for treating insomnia in cerebrovascular disorders, as well as in Alzheimer's and Parkinson's diseases (10).

In conclusion, the repeated administration of aniracetam

TABLE 3

EFFECTS OF PHYSOSTIGMINE AND NEFIRACETAM ON DAILY MOTOR ACTIVITY AND MEALTIME-ASSOCIATED ANTICIPATORY BEHAVIOR IN AGED RATS

Treatment	Dose (mg/kg)	Period	Motor Activity (Counts)		
			Day −1	Day 6	Day 7
Vehicle	—	Light	1759 ± 246	1920 ± 225	1623 ± 209
		Dark	4923 ± 773	4032 ± 396	3920 ± 440
		Total	6683 ± 984	5953 ± 500	5543 ± 579
		L/D ratio	0.39 ± 0.06	0.50 ± 0.07	0.43 ± 0.05
		Anticipation (1130–1330)	3.76 ± 0.99	4.18 ± 0.85	5.28 ± 0.33
Physostigmine	0.1 SC	Light	1591 ± 221	2048 ± 227§	2117 ± 282§
		Dark	4718 ± 625	3207 ± 483#	3308 ± 530#
		Total	6309 ± 792	5255 ± 661§	5425 ± 796§
		L/D ratio	0.34 ± 0.04	0.67 ± 0.06#	0.66 ± 0.04†,#
		Anticipation (1130–1330)	3.26 ± 0.62	5.66 ± 0.32‡	7.92 ± 0.75*,§
Nefiracetam	10 PO	Light	1659 ± 492	1770 ± 257	1769 ± 483
		Dark	4717 ± 719	3324 ± 443	3690 ± 674
		Total	6376 ± 957	5094 ± 659	5460 ± 1129
		L/D ratio	0.36 ± 0.10	0.54 ± 0.06	0.47 ± 0.07
		Anticipation (1130–1330)	2.74 ± 0.53	4.82 ± 0.69	6.22 ± 1.14

Data show means ± SEM obtained from four to six rats/group.

*$p < 0.05$, †$p < 0.01$; significant differences from vehicle (one-way ANOVA followed by Dunnett's *t*-test).

‡$p < 0.05$, §$p < 0.01$, #$p < 0.001$; significant differences from day −1 (repeated measures one-way ANOVA followed by the Ryan-Einot-Gabriel-Welsch test).

restored the mealtime-associated anticipatory behavior impaired in aged rats, which was measured as an index of the circadian time-keeping system, as did physostigmine. The improving effects were independent of a change in appetite or motor ability. Aniracetam was thought to resume the diminished anticipation by ameliorating the timing or temporal regulatory system through a facilitation of the reticulothalamic cholinergic neurotransmission. The evaluation system presented here may be a useful animal model of temporal regulation of behaviors or habitual daily activities.

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