S-20-4  Diurnal Monoamine Variation in Young and Old Rats:  
A Microdialysis Study

A.SANO, K.Aoi, T.AZEKAWA, H.SEI,  
H.SENO, and Y.MORITA

Department of Physiology, School of Medicine, University of Tokushima, 3-18-15 Kuramoto,  
Tokushima 770 (Japan)

I. INTRODUCTION

It is well established that senescence is characterized by a variety of neurochemical and  
morphological changes in the nervous system, resulting to age-related alterations in various  
physiological and behavioral events. Change in sleep-waking patterns is one of the examples.  
There are many documented changes in the amount and patterning of various sleep stages with  
aging [1] [5] [6]. The most striking change is a marked decrease in slow wave sleep [2] [5] [6]. On  
the other hand, there is a significantly increased amount of wakefulness [2] [3] [4] [5] [6]. In  
addition, several authors reported that the circadian organization of the sleep-wake cycle  
apparently changes with increased age [3] [5]. We have also observed the senescence-related  
reduction of diurnal fluctuations in slow wave sleep, paradoxical sleep and wakefulness in rats  
[6].

The sleep-wake mechanism is regulated by concerted interactions between a large number  
of brain regions. Although there are several candidates for the sleep-wake regulating structures,  
the interrelation among them has yet been ambiguous. We have been particularly interested in  
age-related changes of neurotransmitters which may have relevance to the sleep-wake  
regulation influenced by sleep mechanisms per se as well as circadian organization. In the  
present study, using the microdialysis procedure, we investigated the age-related changes in  
extracellular monoamine metabolism in the rat striatum which may participate in the sleep-wake  
regulation.

II. DIURNAL VARIATIONS OF EXTRACELLULAR MONOAMINE LEVELS FROM THE STRIATUM  
in Young Adult and Old Rats

For the study of the neurochemical mechanism involved in sleep-wake regulation, it is  
significant to directly monitor the biochemical changes in relevant to ongoing behavior of  
animals under freely moving situations. From this reason, using on-line and automated  
microdialysis with HPLC-ECD, we continuously monitored extracellular monoamines and their  
metabolites from the striatum of freely moving rats over 24 h at 30 min intervals, together with  
simultaneous polygraphic recordings.

The experiments were performed on two age groups of Wistar male rats. One group  
consisted of rats 2-6 months old, and the other 22-27 months old. Rats were kept under a  
constant light-dark condition (L:D 12:12, lights on at 06:00) with water and food ad libitum. Under  
pentobarbital anesthesia, a guide cannula (0.55mm O.D.) for a microdialysis probe was  
stereotaxically implanted according to Paxinos and Watson’s atlas [7]. Electrodes for  
polygraphic recordings were also implanted. After two weeks of postoperative recovery, the  
microdialysis experiments were carried out. The microdialysis probe was inserted into the  
striatum (A: -0.3, L:3.5, V:4.0-7.2 mm) through the guide cannula, and then at least 3 h later, in  
vivo measurement was started. The dialysis hollow fiber had a molecular mass cut-off of 5,000.  
The dialysis probe was perfused continuously at a rate of 1.5 µl/min with Ringer’s solution  
through a PTFE tube connected to a microinfusion pump. Dialysate was successively sampled  
and automatically injected into the HPLC-ECD system by means of an automatic injector at 30  
in min intervals and analyzed on-line with the HPLC-ECD system.
Fig. 1. The 24 h mean levels of striatal extracellular DA, DOPAC, HVA and 5-HIAA in young adult and old rats. Each value was corrected by the in vitro % recovery of the probe used in the experiment (ranged from 17 to 26%). Filled columns: young adult group, open columns: old group. Vertical lines show SEM. * P<0.05, ** P<0.01 with Student's t-test.

Fig. 2. Diurnal variations of striatal extracellular levels of DA, DOPAC, HVA and 5-HIAA in young adults (A) and old rats (B). The 24 h means of the substances were taken as 100%. Vertical lines show SEM. Shaded regions indicate the dark phase. One-way ANOVA was used to ascertain the rhythms. (A) DA, DOPAC, HVA and 5-HIAA; P<0.01 respectively, (n=6 except for 5 in DA). (B) DA, DOPAC and 5-HIAA; non-significant, HVA; P<0.01 (n=6).

Figure 1 represents the mean levels of striatal extracellular DA, DOPAC, HVA and 5-HIAA in young adult and old rats. 5-HT was not detectable. The levels of striatal extracellular DA, DOPAC, HVA and 5-HIAA significantly decreased in aged rats compared to young adults. In addition, the
magnitude of age-related reduction was greater in dopaminergic than serotonergic substances (-75% in DA, -68% in DOPAC, -71% in HVA and -37% in 5-HIAA). It has been reported that the content and turnover of dopamine decrease with age particularly in the striatum and mesolimbic areas [8]. The present results by microdialysis are parallel with these findings obtained from homogenized tissue. It may be suggested that dopaminergic neurons in the nigro-striatal system is vulnerable to aging.

Figure 2 represents the diurnal variations of striatal extracellular DA, DOPAC, HVA and 5-HIAA levels from young adult and old rats. In young adults, all of the substances showed significant diurnal variations as one-way analysis of variance was used to ascertain the rhythms. The levels of DA, DOPAC, and HVA were maximal at about the middle of the dark phase and minimal at the middle of the light phase. In contrast, the 5-HIAA levels showed the maximal values in the transition period from the dark to the light phase and the minimal values in the transition period from the light to the dark phase. On the other hand, in old animals, none of the above substances, except for the HVA, exhibited significant diurnal variations. The levels of HVA in old rats changed in the similar pattern to those of young adults during the light-dark cycle.

Our present study demonstrated that the diurnal variations in striatal extracellular monoamines were also reduced in aged rats. Such reduction in diurnal variations of striatal extracellular monoamines in aged rats seems to be one factor which underlies age-related changes of rest-activity and/or sleep-wake rhythm. Further studies are needed in order to prove this point.

III. EFFECT OF HOUSING IN AN ENRICHED ENVIRONMENT ON AGE-RELATED CHANGES OF STRIATAL EXTRACELLULAR MONOAMINES

Housing in an enriched environment has been demonstrated to have a variety of effects in rodents [9]. These effects ranged from neurochemical, physiological, anatomical to such behavioral changes as learning ability, sleep-waking patterns, etc. We also investigated whether or not differential housing could affect age-related changes of rat striatal extracellular monoamines. Rats were randomly assigned to either the enriched condition (EC) or the isolated condition (IC) at 22 months old. The EC rats were housed socially, 10 per a large cage (70x70x46 cm), made of wire mesh, containing a variety of stimulus objects. The IC rats were individually housed in a small cage (20x30x20 cm). After at least 2 months environmental exposure, microdialysis experiments were performed according to the above-mentioned procedures.

Fig. 3. Time course of DA, DOPAC, HVA and 5-HIAA levels over 24 h from the striatum in enriched environment-housed (open circle) and isolatedly-housed (closed circle) rats under a constant light-dark condition (L:D 12:12, lights on at 06:00). The data represent the mean and SEM (vertical bars) of rats. The differences between EC and IC group in extracellular levels of DOPAC were statistically significant (*P<0.05, Student's t-test after two-way ANOVA). The time course variations in DA, DOPAC, HVA and 5-HIAA were not significant in any of the housed cases.
As shown in Fig. 3, the EC rats showed a significant increase of the levels of striatal extracellular DOPAC throughout the dark phase, compared to the IC rats. Such increase of DOPAC in the EC rats seems to be parallel with the findings that environmental enrichment results in beneficial consequences on locomotor recovery following cortical lesions [10]. DA, HVA, and 5-HIAA did not exhibit significant differences between the EC and the IC group. The senescence-related changes of diurnal rhythm in the striatal extracellular DA, DOPAC, HVA and 5-HIAA were not affected by differential housing exposure.

IV. SUMMARY

The levels of striatal extracellular DA, DOPAC, HVA and 5-HIAA significantly decreased in aged rats compared to young adults. The magnitude of age-related reduction was greater in dopaminergic than serotonergic substances. The levels of striatal extracellular monoamines in young adult rats showed significant diurnal variations. On the other hand, these substances of aged rats, except for HVA, did not exhibit significant diurnal variations. Enriched environment housing increased levels of striatal extracellular DOPAC in aged rats, but did not influence on the age-related changes of diurnal variations in the striatal extracellular monoamines.

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