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BEHAVIORAL STUDY OF ULTRADIAN ACTIVITY PERIODS OF OF1 MICE ENCLOSED IN EXPERIMENTAL CAGES OF DIFFERENT DIMENSIONS

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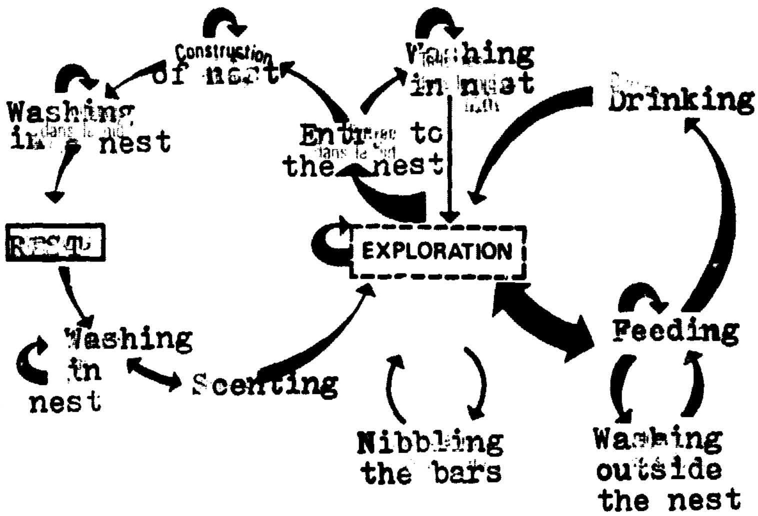
rarely associated (taking of food and construction of the nest; exploration and washing at the end of activity). The most frequent succession of behaviors is described in Figure 2, presented in the form of a cycle based upon the same behaviors of cleaning of the face and body.



The thickness of the arrows is proportional to the frequency of the sequences for each behavior considered.

↷ = Behavior carried out continuously for several minutes.

Figure 2. Diagram of the succession of behaviors during ultradian activity periods during diurnal phase.

<u>Linear Correlations Between Behavioral Frequencies, Durations of Activity, and Intervals of Rest</u>

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Summary /295*

Male OF1 mice are enclosed in cages of different dimensions (cage A = 23 x 8 x 8 cm, cage B = 36 x 27 x 17 cm), in an alternating LD regimen, at an ambient temperature of 22-23°C. The successions of the behavioral sequences of ultradian activity periods are noticed by direct observation during 11 following hours in L.

The experimental situation modifies the mean duration time and the behavioral organization of each activity period. However, the comparison of the overall activity time lengths and the comparison of the overall behavioral frequencies suggest that the energy spent per mouse is constant whatever is the experimental cage.

Introduction

Direct observation for 11 consecutive hours of male OF1 mice placed in an alternating LD regimen (12:12) reveals the existence of an ultradian activity rhythm and resting rhythm. This behavioral rhythm, cited within some older studies, to our knowledge has not been /296 the object of systematic investigations.

Since a previous study has shown the influence of the experimental situation on the behavior of isolated or grouped animals [7], one will be interested here in the effects of the size of the cage in which the animal is observed in order to state precisely if the duration and behavioral organization of ultradian periods remain stable regardless of the experimental conditions.

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*Numbers in the margin indicate pagination in the foreign text.

L = LIGHT; D = DARK.

Procedure

Subjects

Four 6-week-old male OF1 mice (IFFA CREDO Oncins), in an alternating LD regimen (12:12) for 2 weeks, were enclosed alone in polystyrene cages measuring 23 x 8 x 8 cm (cage A) for four days, then in polycarbonate cages measuring 36 x 27 x 17 cm (cage B) for four days.

Four other male OF1 mice of the same age, in an alternating LD regimen (12:12) for two weeks, were enclosed alone first in cages B for four days, then in cages A for four days.

The results therefore concern eight males enclosed within cages A with an area of 184 cm^2, and the same eight males enclosed within cages B with an area of 972 cm^2.

The animals are provided with a litter (sawdust), nourishment (food UAR), and drink (fresh water) *ad libitum*, and with a cotton ball for construction of a nest. /297

Experimental Conditions

The illuminated phase is produced by the functioning of white flourescent tubes (100 lux) from 0600 to 1800 hours. From 1800 to 0600 hours red flourescent tubes (10 lux) create a dark phase, and permit eventual observations during the active period. The temperature of the observation room was maintained at 22-23°C.

Protocol

Observations were made on the fourth day in both experimental situations. It is a question of direct observations of four males for 11 continuous hours (from 0800 to 1900 hours), consisting of 10 hours during the diurnal phase and 1 hour during the nocturnal phase. Cages were separated from the observer by a transparent glass partition, and separated from each other by opaque partitions. Successions of behavior were surveyed for one-minute periods. The behavioral sequences chosen are the following:

Coding Washing In The Nest	Behavioral Sequence
DEB (*)	Washing of the face and body before excursion outside the nest (prior to activity)
FIN (*)	Washing of the face and body after the last return to the nest (after activity)
PDT	Washing of the face and body during activity
HS	Brief washings of the face outside the nest
EXPLO	Exploratory movement
ALIM	Taking of nourishment from the feeding trough
BOIS	Taking of drink from the feeding bottle
NID	Construction of the nest
ENT, NID (**)	Entry into the nest
FLAIR (**)	Scenting: animal is immobile and scents, head raised
MOR BAR (**)	Nibbling the bars of the cage or of the feeding trough
REPOS (**)	Immobility in the nest, eyes closed

*The results concerning these behaviors correspond to their duration, estimated through surveys at one-minute periods, seeing that these behaviors are carried out uninterrupted for several minutes.

**These behaviors will not occur except during study of the behavioral organization of an activity period.

Results

Ultradian Activity Period

Number of Activity Periods from 0800 to 1900 Hours (Figure 1)

The number of activity periods varies from 5 to 8. Four out of eight mice develop more activity periods in cage B.

Mean Duration and Distribution of Activity (Figure 1)

The results deal with complete activity periods. Mean duration: cage A:40.2 min. (s.d.=25.4), cage B:25.9 min. (s.d.=10.3). The difference between the two means is statisti- /298
cally significant (t=2.99, d.f.=7).

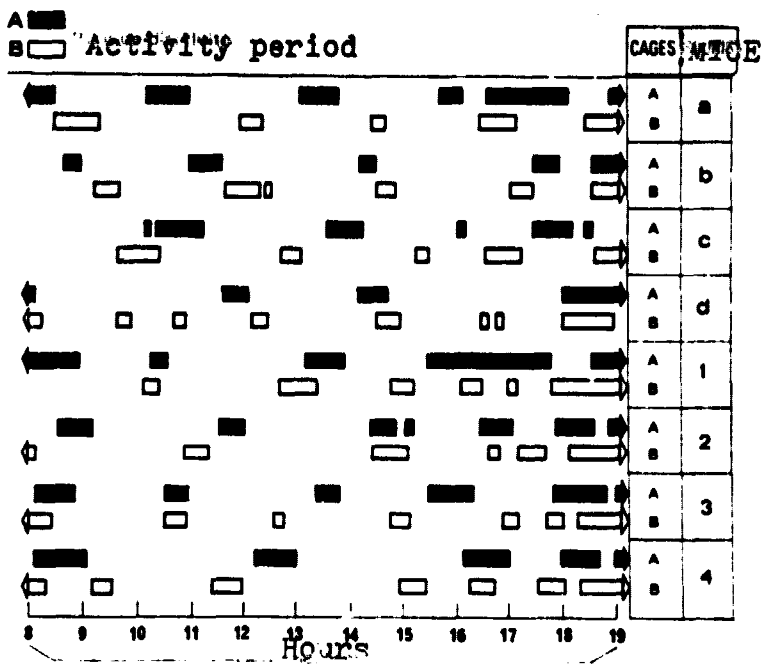


Figure 1. Distribution and duration of ultradian activity periods in diurnal phase.

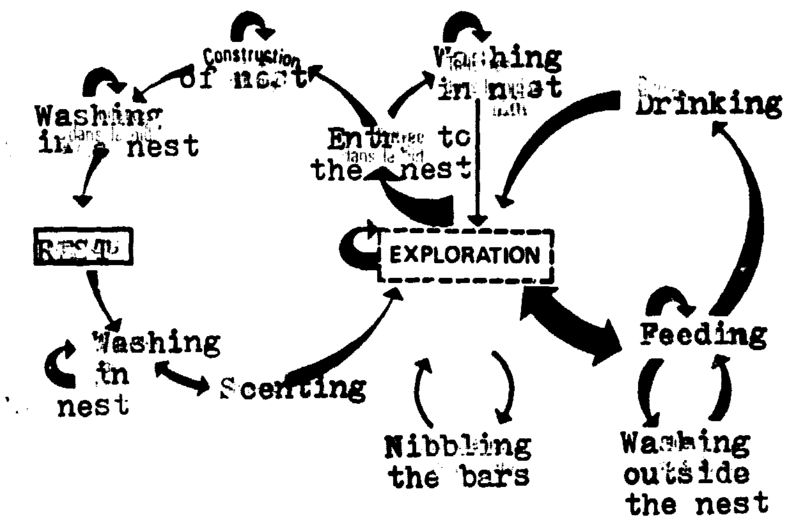
The mean duration of rest intervals in cage A is 93.5 min. (s.d.=56); that of the first rest interval is 118.2 min. (s.d.=58), that of the last is 36.5 min. (s.d.=24).

In cage B the mean duration of rest intervals is 86.6 min. (s.d.=46.4); that of the first interval is 120.7 min. (s.d.=34), that of the last is 50.6 min. (s.d.=26).

In both cases the frequency of activity periods increases at the end of the diurnal period. Observation carried on one hour after the white light is turned off indicates likewise that relative darkness does not immediately stop the activity of most of the mice.

Behavioral Organization

An analysis of the behavioral structure of ultradian activity periods has been accomplished by calculating the matrices of the most frequent successions of behavior observed during 36 total periods. The results indicate that behavioral organization is the same in both situations. Certain behaviors were never or /300 rarely associated (taking of food and construction of the nest; exploration and washing at the end of activity). The most frequent succession of behaviors is described in Figure 2, presented in the form of a cycle based upon the same behaviors of cleaning of the face and body.



The thickness of the arrows is proportional to the frequency of the sequences for each behavior considered.

- Behavior carried out continuously for several minutes.

Figure 2. Diagram of the succession of behaviors during ultradian activity periods during diurnal phase.

Linear Correlations Between Behavioral Frequencies, Durations of Activity, and Intervals of Rest

Correlations were calculated on the frequencies correspond-

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ing to each complete activity period. The test used is the calculation of coefficients of the ranges of Kendall (Table 1).

TABLE 1. LINEAR CORRELATIONS BETWEEN BEHAVIORS, DURATION OF ACTIVITY AND OF REST IN SMALL CAGE (A) AND LARGE CAGE (B)[1,2] /299

Small cage (A) N=29 T > 0.257

		Washing										
		DEB	HS	FIN	D + F[3]	EXPL	ALIM	BOIS	NID	DU[3]	IP[3]	IS[3]
Washing	DEB				0,51				— 0,28			
	HS					0,64	0,40	0,39		0,56		
	FIN	— 0,26	0,31		0,62						0,41	0,28
	D+F	0,29	0,26	0,47							0,39	
	EXPL		0,46	0,43	0,35		0,55	0,63		0,83		
	ALIM		0,40	0,35	0,28	0,69		0,61	— 0,51	0,58		— 0,35
	BOIS		0,36	0,36	0,26	0,79	0,67			0,63		
	NID	— 0,26	0,41	0,52	0,33	0,45	0,30	0,42				0,39
	DU		0,52	0,45	0,67	0,60	0,51	0,51	0,49			
	IP		0,28	0,42	0,37				0,36	0,30		
	IS	— 0,29		0,52					0,45		0,39	

Large cage (B) N = 34 T > 0.235

[1]Test of coefficient of Kendall ranges. Only significant results ($p < .05$) have been entered.

[2]Commas in tabulated material are equivalent to decimal points.

[3]D + F = DEB + FIN; DU = duration of activity period; IP = duration of rest period preceding activity period; IS = duration of rest period following activity period.

Total Observation Period

Overall Activity Time Lengths During 11 Hours (In Minutes)

Comparison of the overall activity time lengths, carried out by paired series, shows that mice are active just as long in cage A as in cage B ($t = 1.39$, d.f. = 7). The difference of mean duration time in the longer periods reported therefore compensates for the difference in the number of activity periods between the mice in cages A and B.

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TABLE 2. OVERALL ACTIVITY TIME LENGTHS DURING 11 HOURS (IN MINUTES)

	1	2	3	4	a	b	c	d
Cage A	285	197	210	196	225	144	155	151
Cage B	215	184	183	222	172	145	143	176

Overall Behavioral Frequencies

TABLE 3. SUM OF THE FREQUENCIES OF BEHAVIORS DURING 11 HOURS IN CAGES A AND B

	TDN	TOI	DEB	PDT	HS	FIX	EXPL	AHB	BOI	NID	DUR	Mice
Cage A	83	6	54	0	41	29	144	24	16	14	285	1
	97	0	55	1	16	41	72	32	10	31	197	2
	95	0	53	6	22	36	115	37	23	24	210	3
	106	0	58	5	17	43	83	19	9	11	190	4
	44	0	23	3	37	18	111	23	19	15	225	a
	82	0	18	9	20	55	39	14	2	31	144	b
	65	15	28	4	26	18	85	5	8	33	155	c
	49	13	17	4	16	15	58	9	16	37	151	d
Cage B	98	7	44	8	20	30	92	28	13	23	215	1
	96	13	41	3	9	39	65	16	14	17	184	2
	105	25	47	0	13	33	50	23	9	20	183	3
	161	0	97	8	8	56	66	34	0	14	222	4
	70	0	27	1	21	42	69	17	17	17	172	a
	84	0	33	2	5	40	53	21	8	22	145	b
	90	16	41	2	7	31	43	19	10	21	143	c
	99	28	32	3	10	36	59	26	18	18	176	d
Student t	3.30 (*)	2.47 (*)	1.22	0.38	6.72 (*)	2.34 (*)	2.83 (*)	0.60	0.30	1.72	1.40	

(*) Student t test on paired series significant at $p < 0.05$.

TDN = sum of the washings in the nest; TOI = estimated duration of the activity periods composed only of washings; DUR = duration of the activity (in minutes).

Discussion

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The results therefore show that the ultradian activity periods of the diurnal phase present a similar succession of behaviors, and that the animals organize their activity differently according to the experimental situations. A cage with a more restricted volume and area brings about a longer duration of activity periods and undoubtedly in consequence a different connection of behaviors among them.

Indeed, in cage B the duration of ultradian activity, linked to the preceding rest interval and for the most part to the behavioral frequencies, appears to express an organized situation. The duration of washings prior to and after activity varies in inverse fashion, which maintains a better connection of the total duration of the activity and that of the preceding rest interval. These correlations, the last of which is found again in the situation in cage A, could be interpreted as a participation of thermoregulation on the washing behaviors.

However caution should be used as to the function of washings prior to activity. They were never connected to the duration of the activity and have a negative connection with the frequency of construction of the nest. These facts could exclude them from all participation in the regulation of the temperature of the animal, contrariwise then to washings following activity.

The less numerous correlations between the different behaviors appears to express a less organized situation. This could come from an emotional reactivity, more important when the size of the cage is restricted [8]. The increase of substituted activities (brief washing of the face [9]) could also confirm it. In cage A rest time following activity is shorter as the frequency of taking of food increases. The animal therefore activates itself all the more rapidly as its accumulated supplies are all the more numerous. This result was never found in cage B; it is possible that in a cage of restricted size, the animals only organize their activity around the taking of nourishment, whereas in a larger cage their activity is a function of all behaviors.

The positive connection between the frequency of movements of construction of the nest and the duration of rest intervals following activity, found in both situations, in noteworthy on the one hand because the duration of rest following activity is thus predicted, and on the other hand because it suggests the role (similar to that of washing in the nest) of thermoregulation: indeed, the animal arranges its nest all the more as more time passes without activity, during which time its body temperature decreases. The positive connection between exploratory frequency and that of taking of food and of drinking was found equally in both situations, a connection furthermore pointed out in *Mus musculus* [10] and *Peromyscus maniculatus* [5].

When the investigation on the total activity of the animals during 11 consecutive hours was completed, one reports that the duration of that activity is the same, regardless of the experimental situation. Besides, investigation of the overall behavioral frequencies shows that the animals reacted to changing situations through a modification /302 of the number of exploratory excursions, more numerous in a restricted cage. The frequency of taking of food, of drinking, and of construction of the nest, did not undergo and modification; it is possible that the mice increase their locomotive activity in cage A or decrease it in cage B in order to maintain an identical level of activity. Indeed, a locomotive excursion in cage B could represent a more important expenditure of energy than an excursion in cage A, the distance traversed being greater in the former case.

Washings in the nest have a more significant total duration time in cage B, although washings outside the nest are more frequent in cage A. The animals therefore have a tendency to reduce the duration of washings in the nest when exploration is more important, bringing about a greater frequency of washings outside the nest, with a regulation of the total duration time of this cleaning behavior.

The preceding analyses suggest therefore that the observed differences in the two cages could be due to a reaction or adaptation of the animals to the situation by taking into account constant expenditure of energy.

Conclusion

The manner of surveying behaviors used here therefore permits us to state that the mean duration time of ultradian activity periods and their behavioral organization varies according to the experimental situation. The mice also modify exploratory frequency and the duration of certain washing actions as a function of the size of the cage, but maintain the same occurrence of most other behaviors. Interpretation of these results indicates a constant expenditure of energy by the animal within the time period considered. Further accounts of variables linked to energetic metabolism could permit a better understanding of the underlying mechanisms.

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