Part Two

AGEING OF THE CIRCADIAN SYSTEM AND MEMORY

Chapter 5

Circadian Patterns of Behaviour in Young and Aged Rats and Mice

Barbara A.M. Biemans

Scope: *In this chapter, behavioural changes of the circadian system that occur with ageing are presented in three strains of rats and mice that have been studied in this thesis. For an overview of literature on circadian changes with ageing, I refer to chapter 1. Results presented here were assembled over the course of four years. The tables and graphs contain information about age-related changes in wheel running, feeding and general activity of Wistar Unilever and Fischer 344×Brown Norway (F×BN) rats, and CBA/ca house mice.*

Housing and light conditions

Animals were individually housed in light and temperature controlled climate rooms. Rats were kept in cages (width × depth × height: 30×46×50 cm) equipped with a running wheel (∅ 25 cm) (Fig. 1A). Two switch closures were recorded in with every turn of the wheel. A PC-based event recording system (ERS) stored events in 2-minute bins. Feeding behaviour was recorded by means of a food hopper, suspended outside the cage. When a rat attempted to obtain food pellets inside the food dispenser, this activated a switch. General activity was measured by passive infrared (PIR) detectors. Besides general locomotor activity, PIR measurements also reflect wheel running and feeding activity. Mice were individually housed in smaller wheel running cages (width × depth × height: 10×30×13 cm) (Fig. 1B). The turn of a wheel (∅ 14 cm) resulted in two activity pulses.

Rats and mice were either entrained to 12:12 hours light-dark (LD) cycles, or released in free-run under constant dim red light (DD). During the light phase, fluorescent lamps (Philips) were used with a light intensity inside the cage of ± 25 lux. Relatively low light levels were used to reduce degeneration of the visual system due to high light intensities. This was especially relevant since we studied ageing, and rats were thus kept in the laboratory for a long time. This same light intensity was used in all the experiments. During the dark phase of the LD cycle and during DD, the room was dimly lit by two red-painted light bulbs (Osram, 25 Watt), resulting in a light intensity of about 0.5 lux inside the cage.

Chronology of studied groups of rats and mice

		*group/ age**	*Recorded behaviour*
Apr- May 2001	Wistar Unilever rats	- 4 - 26	- feeding - wheel running
Oct-Dec 2000	F×BN rats first group	- A_{10} - B_{31}	- feeding - wheel running
Jan 2001- May 2002	F×BN (data not shown)	- $A_{(10\text{-}28)}$	- feeding - wheel running
May- June 2002	F×BN second group	- C_4 - A_{28}	- feeding - wheel running
July- Oct 2002	CBA/ca mice	- 4 - 17	- wheel running

*A_{10} indicates experimental group A studied at 10 months of age. A_{28} indicates same group studied at 28 months of age.

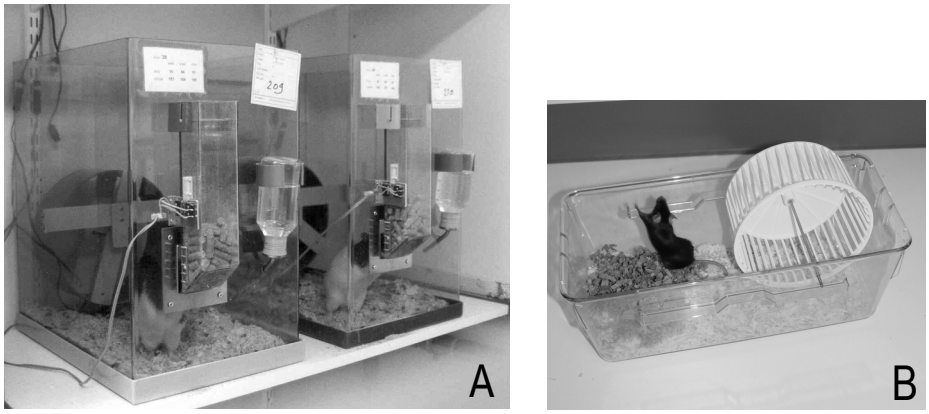

Figure 1. Rat (A) and mouse (B) behavioural recording cage

Data analysis

For the light phase, average total daily activity, and the percentage of time active during the light phase were calculated. Altered distribution of activity may be an indication of a changed organisation of the circadian system. In addition, the amplitude (Amp) of the rhythm was calculated as follows: Amp = h'-l', where h' is the highest hourly value of $a/\bar{a}$ of the day, and l' is the lowest. a = activity per hour in the average daily curve, and $\bar{a}$ is the average of the hourly values of a.

In DD, freerunning periods (τ) were calculated by means of chi-square periodogram analysis (Sokolove and Bushell, 1978). The periodogram was calculated on qualitative data (activity in 2-min intervals scored as 1; absence of activity as 0), and yielded significant frequencies and their relative strength (ΔQp). Variability in rhythmicity was analysed based on onset, offset, and centre of gravity of activity. Onset and offset of activity were calculated as follows (see also Daan and Oklejewicz, 2003): a running mean (RM) of 48 hours was calculated over the raw data to determine baselines values, but allowing for changes in activity over time. The intercepts between this baseline and either raw values, a 1-hour running mean, or a 3-hour running mean were determined. These were taken as on- and offsets of activity. An example of such a computation is given in Figure 2. On the basis of these on- and offsets, the activity period (α) was calculated (offset minus onset). The choice for using raw data, 1-h or 3-h RM for further calculations depended on whichever measure yielded the lowest variation in most of the young animals, which turned out to be the 3-h RM. The total daily activity, corrected for τ where necessary was also determined. The results for the different strains are given in tables.

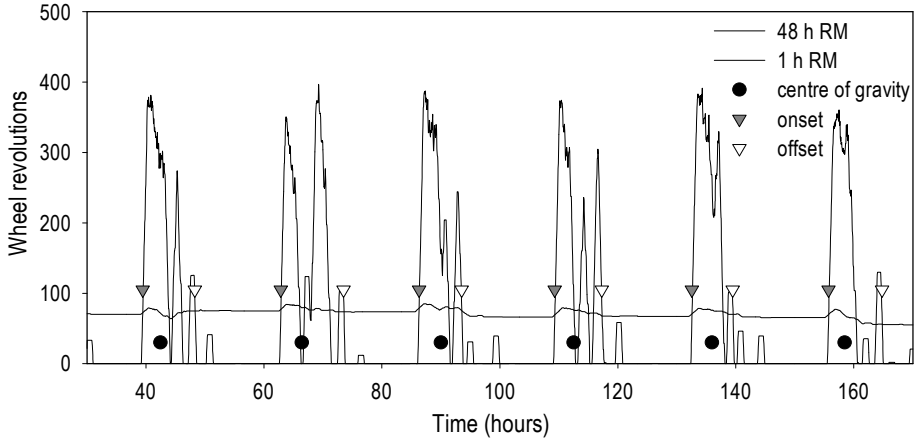

Figure 2. Example of determination of onset (grey triangle) and offset (white triangle) based on intercept between 48 hour RM and raw data, 1-h RM or 3-h RM. This example shows the 48 RM and 1-h RM. Centre of gravity is also indicated (black dot).

Rattus norvegicus

Wistar Unilever

Wistar rats exhibit a clear age-related change in circadian organisation of behaviour. Rhythms of the old rats are more fragmented and activity is more scattered over the day than in young rats, with increased daytime feeding. Figure 3 shows example double-plotted actograms of feeding behaviour for two young (4 months) and two aged (26 months) rats. The average pattern of feeding in LD conditions is shown in Figure 4. Young rats have two distinct bouts of high activity, with the highest feeding bout at the end of the dark period. This is consistent with the offset being more precise than the onset (Table 1). The centre of gravity of activity is the phase marker least variable from day to day, in young as well as aged rats.

Aged rats usually have three bouts instead of two, which are lower than the two bouts in the young rats. Young rats did not eat more, i.e. they did not use the food hopper significantly more often than the aged rats. It is conceivable, however, that aged rats ate less, but had more trouble using the feeder, and had to make a bigger effort to obtain the same amount of food. The opposite (less effort to and thus get the more pellets) is not very likely. Alternatively, they could obtain the same amount, and leave it on the floor without eating it. However, the aim of this study was not to determine food intake, but to measure the circadian pattern of feeding,

and this could successfully be done (Fig. 4). Wheel running was hardly observed with the aged rats (Table 1).

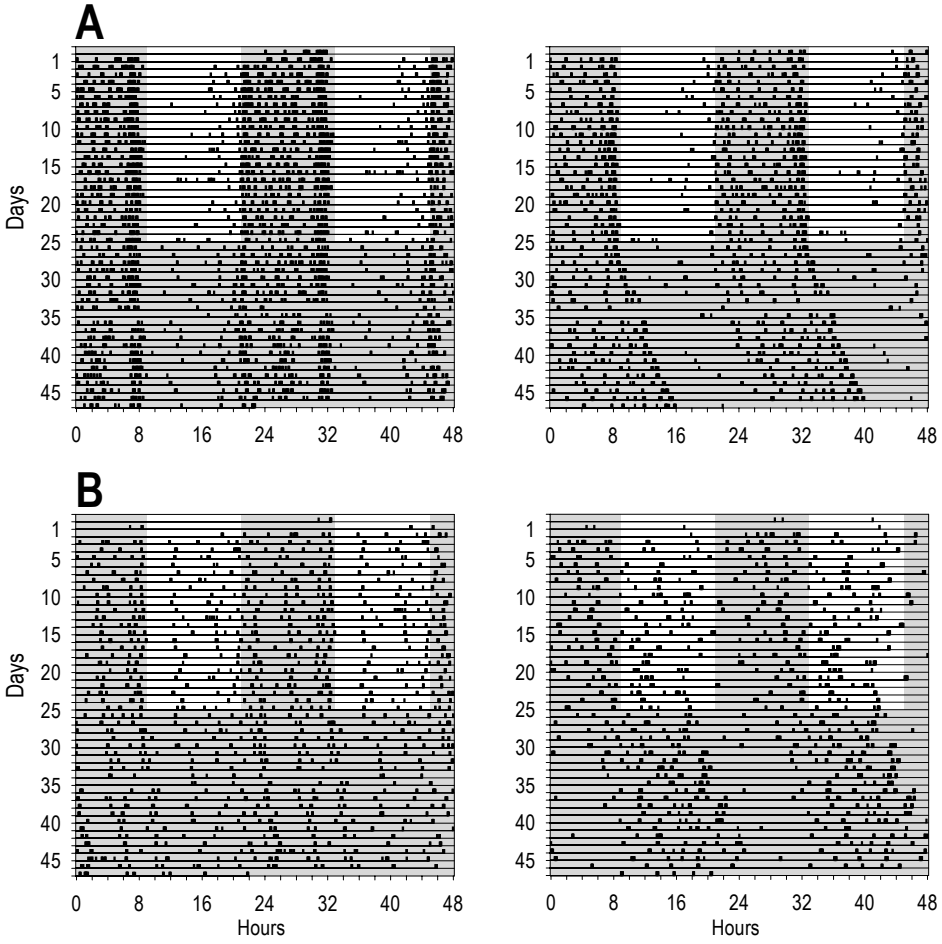

Figure 3. Double-plotted actograms of feeding activity in Wistar Unilever rats. **A .** Examples of two 4 months old **B.** Examples two 26 months old rats

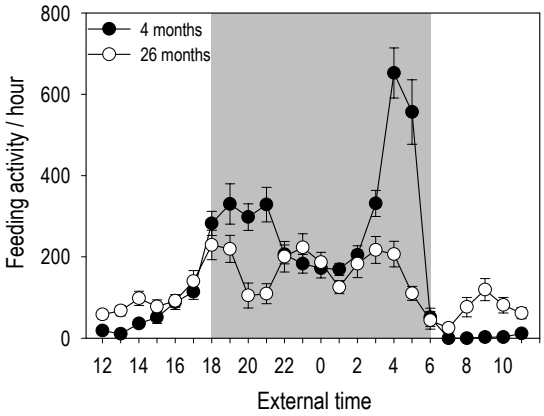

Figure 4. Feeding activity in Wistar Unilever rats

Table 1. Circadian feeding and wheel running behaviour in Wistar Unilever rats

	FEEDING		WHEEL RUNNING	
	4 months	26 months	4 months	26 months
	n=17	n=13	n=17	n=13
Light-dark cycle (12:12 hrs)				
Daily activity (16-day avg.)	4102 ± 346	3060 ± 332	894 ± 182***	27 ± 10
Amp	4.29 ± 0.14****	2.72 ± 0.15	n.d.	n.d.
% Activity in light phase	9.7 ± 0.7****	31 ± 2.5	n.d.	n.d.
Constant darkness (dim red light)				
Daily activity (11 days)	2053 ± 178	1687 ± 153	580 ± 107	n.d.
τ	24.25 ± 0.04**	24.07 ± 0.04	24.34 ± 0.04	n.d.
ΔQp	312 ± 24***	91 ± 11	332 ± 36	n.d.
α	14.0 ± 0.18****	16.1 ± 0.36	11.7 ± 0.46	n.d.
Variation onset (3-h RM)	1.57 ± 0.12***	3.39 ± 0.42	1.44 ± 0.25	n.d.
Variation offset (3-h RM)	1.27 ± .17**	3.23 ± 0.49	2.5 ± 0.14	n.d.
Variation centre of gravity	1.16 ± 0.07**	2.86 ± 0.47	1.05 ± 0.14	n.d.

Amp = amplitude (definition: see text), τ = freerunning period, ΔQp = relative strength of strongest significant circadian period, α = activity period, RM = running mean, ** p<0.01, *** p<0.001, **** p<0.0001, n.d. = not determined.

Fischer 344 × Brown Norway f1 (F×BN)

In this rat strain, changes comparable to those in the Wistar rats were observed. Figure 5 shows examples of feeding, wheel running and PIR actograms. Two groups of these rats were studied. The first group consisted of twenty 10-months old (A10) and twenty 31-months old (B31) rats, of which feeding and wheel running was measured (Fig. 5A+B). The second group consisted of nine rats from the first group, and newly bought rats. The rats from the first group (A28) had been monitored from the moment they came into the laboratory onward, and reached the age of 28 months at the time of this recording period. The young rats in this second group were 4-6 months old at the time of the experiment (C4). Wheel running, feeding and PIR was measured in this second group (Fig. 5C-E). Neither aged nor young rats spent much time in the running wheel (Table 2). Therefore, wheel running was not used for further calculations other than total activity during LD.

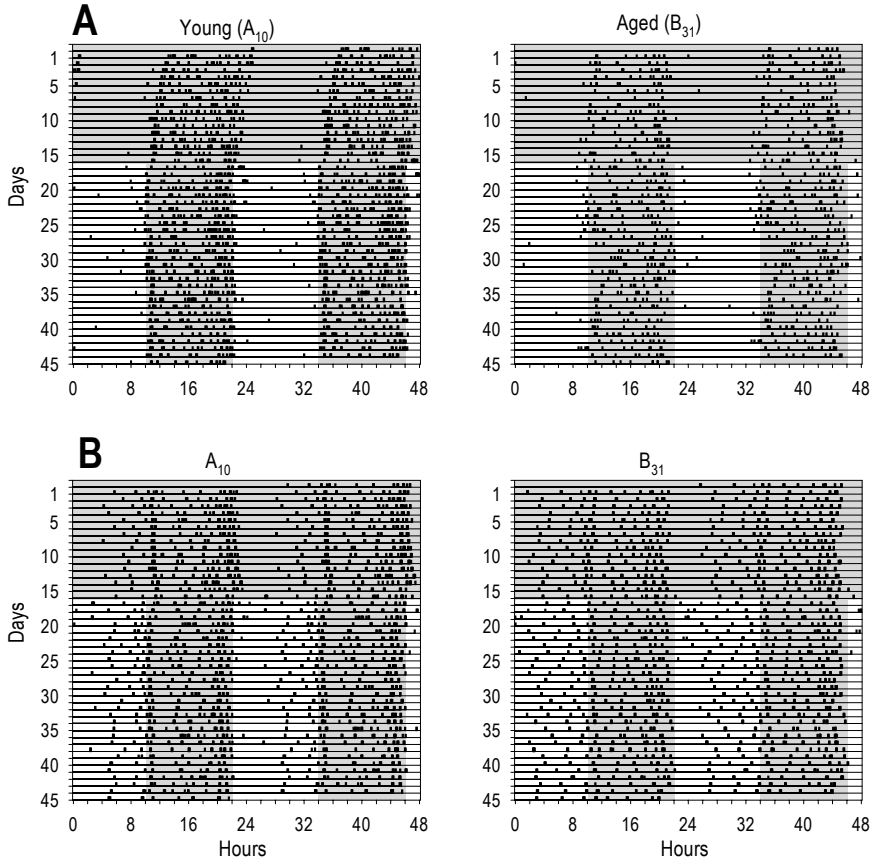

Figure 5 A-B. Double-plotted actograms of circadian rhythms in F×BN rats. **A.** 1st group: Wheel running, best runners. **B.** 1st group: Examples feeding behaviour.

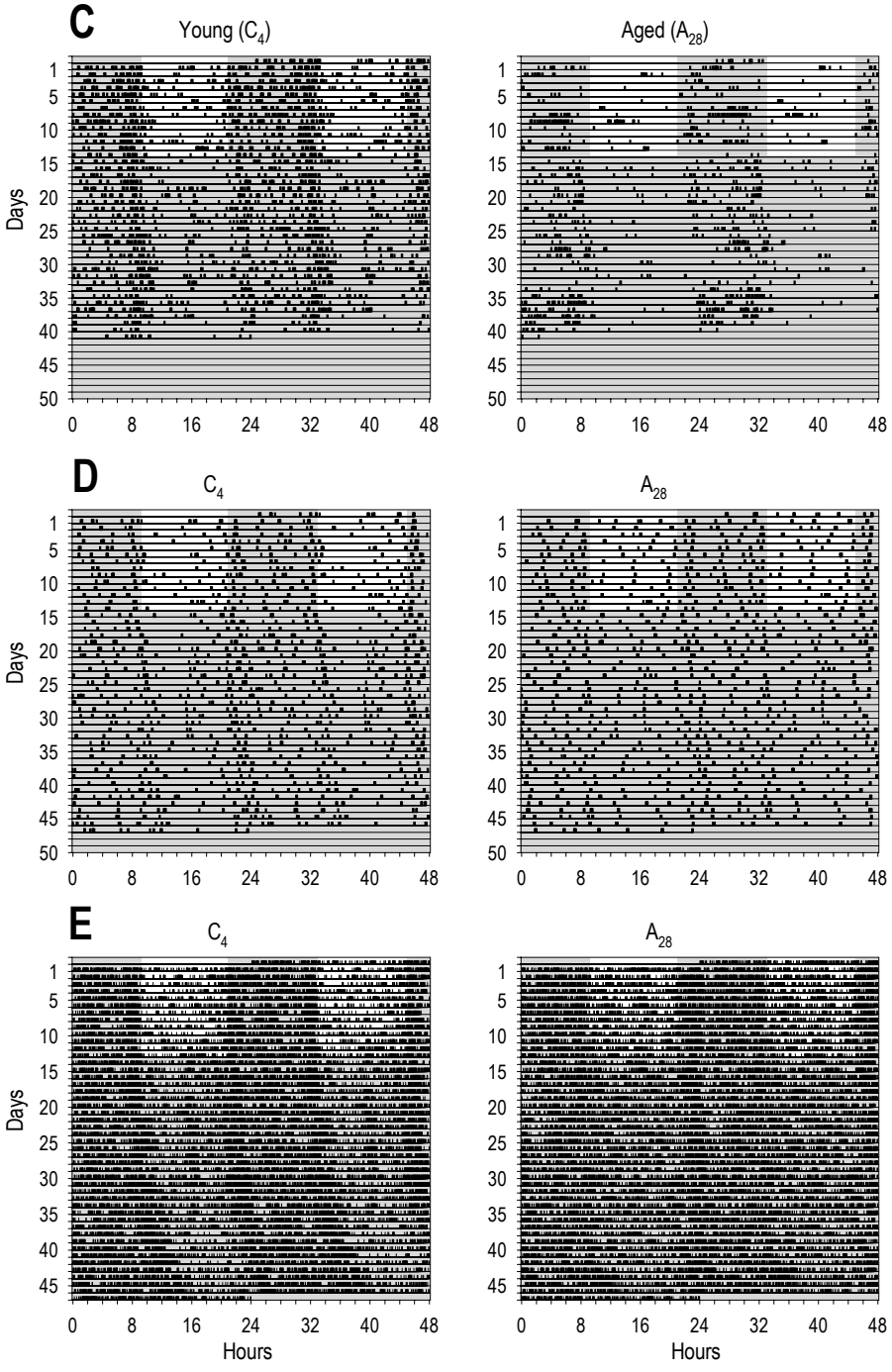

Figure 5 C-E. Double-plotted actograms of circadian rhythms in F×BN rats. **C.** 2nd group: Wheel running, best runners. **D.** 2nd group: Examples feeding behaviour. **E.** 2nd group: Examples general activity (PIR).

First group

Figure 6 shows average patterns of feeding behaviour for the first group. Both young and aged rats have two clear activity bouts during the dark phase, with a lower bout in the middle of the dark phase. The first peak, after transition from light to dark, is more pronounced in F×BN than in the Wistar rats. Aged rats of the F×BN (B31) strain also spent a higher amount of their time feeding during the light phase (Table 2), but the difference is less dramatic than in Wistar rats.

Drinking was measured in F×BN rats as part of an experimental procedure (by weighing drinking bottles at the same time of day for 4 days), and it was found that the aged rats (B31) drank more than the young rats (A10). This is in contrast with literature, which states that this particular strain drinks less with age. It might be that severely aged individuals, such as these, drink more that moderately aged ones as used in those studies (24 months; Silver *et al.*, 1993). Excessive drinking was specifically observed in a few individuals just prior to death.

Table 2. Circadian feeding and wheel running behaviour in F×BN (f1) rats.

1st group	FEEDING		WHEEL RUNNING	
	10 months (A_{10})	31 months (B_{31})	10 months (A_{10})	31 months (B_{31})
	N=20	n=20	N=20	n=20
Light-dark cycle (12:12 hrs)				
Daily activity (12 days)	2444 ± 103	2096 ± 275	41 ± 15	12 ±3
Amp	3.7 ± 0.17**	3.0 ± 0.16	n.d.	n.d.
% Activity in light phase	24.5 ± 0.8***	31.8 ± 1.5	n.d.	n.d.
Constant darkness (dim red light)				
Total daily activity (14 days)	2846 ± 183	2547 ±199	n.d.	n.d.
τ	24.0 ± 0.02*	23.9 ± 0.04	n.d.	n.d.
ΔQp	284 ± 17****	151 ± 18	n.d.	n.d.
α	14.4 ± 0.15*	15.2 ± 0.26	n.d.	n.d.
Variation onset (3-h RM)	2.1 ± 0.14*	2.52 ± 0.16	n.d.	n.d.
Variation offset (3-h RM)	1.6 ± 0.26*	2.77 ± 0.30	n.d.	n.d.
Variation centre of gravity	1.82 ± 0.13**	2.38 ± 0.14	n.d.	n.d.

Amp. = amplitude (definition: see text), τ = freerunning period, ΔQp = relative strength of strongest significant circadian period, α = activity period, RM = running mean, * p<0.05, ** p<0.01, *** p<0.001,**** p<0.0001, n.d. = not determined.

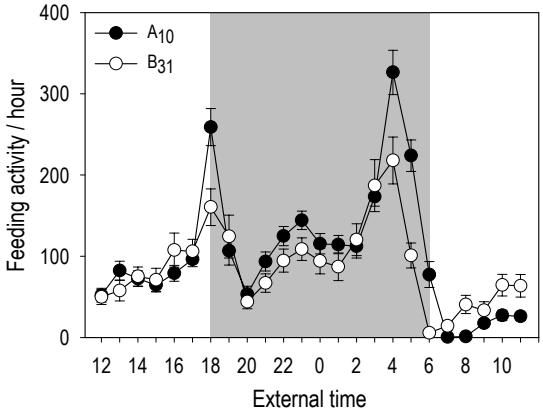

Figure 6. Feeding activity in F×BN rats (1st group)

Second group

The young rats in this second group (C4) were younger than the young rats in the first group (A10) (4-6 months versus 10-11 months), and the aged rats (27-29 versus 31-32 months) as well. This has to be kept in mind when comparing these data. Figure 7 shows feeding (7A) and general activity (7B) in LD, plotted over the 24 h cycle. The pattern looks somewhat more disordered than in the previous group, but this group is smaller than the first. Again, two clear peaks at the beginning and the end of the night are discernible, but the mid-dark peak is more pronounced in this group than in the first F×BN group. Also, the older rats (A28) show a different phase relationship to the Zeitgeber, as they are phase-delayed compared to the young rats (C4). This is the case for both feeding and PIR. In the first group, this was not the

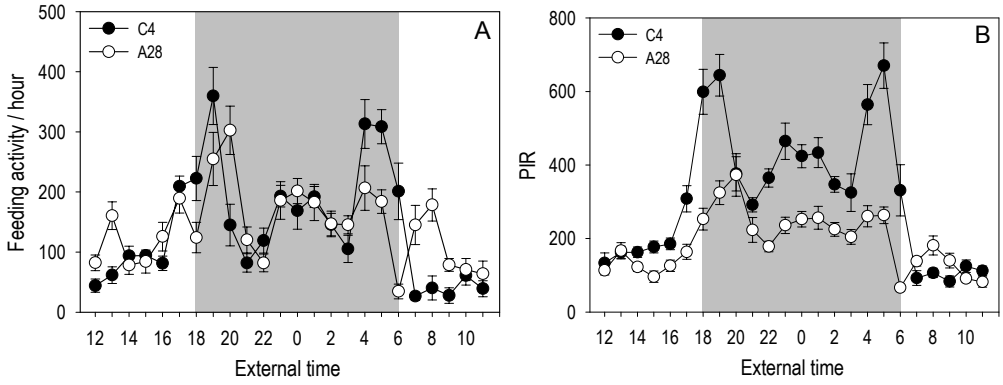

Figure 7. F×BN rats (2nd group) A. Feeding activity B. General activity.

case, as the hourly activity peaks in feeding of A10 and B31 coincided at the onset of darkness (Fig. 6).

General activity (PIR) was significantly decreased in aged rats (Table 3) but still enough present to suggest that their locomotor capacities were largely intact. Wheel running is possibly too much effort for the aged rats, although young individuals did not feels much need to exercise in the running wheel either (Total daily activity in LD young: 239±85; aged: 12±5). The wheel could have been slightly too small for aged rats.

Within individual comparison (A10 vs. A28)

Differences in output of behaviour are present even within a single strain. Interesting are the young ones of the first group, as these can be compared to themselves at a higher age (Fig. 8A). The activity patterns of the young F×BN rats from the first group (A10), bear more resemblance to those of the aged rats in the same experiment (B31) than to their own at a later age (A28) (more pronounced midnight peaks). This suggests that it had something to do with experimental conditions. It could be explained by the scheduling of the light dark cycle: in the first group, the rats were kept on a reversed LD cycle, and were checked regularly during their active period. Disturbing the rats during the night could have led to suppression of feeding activity. In the second group, rats were left undisturbed during their active phase in most cases. Their circadian system deteriorated over time in some aspects (decreased amplitude, more daytime feeding, higher variation), but in other they "improved" (higher feeding activity during LD). They also changed their phase relationship to the Zeitgeber, which cannot really be explained age, as the even older individuals (B31) do not show a delay.

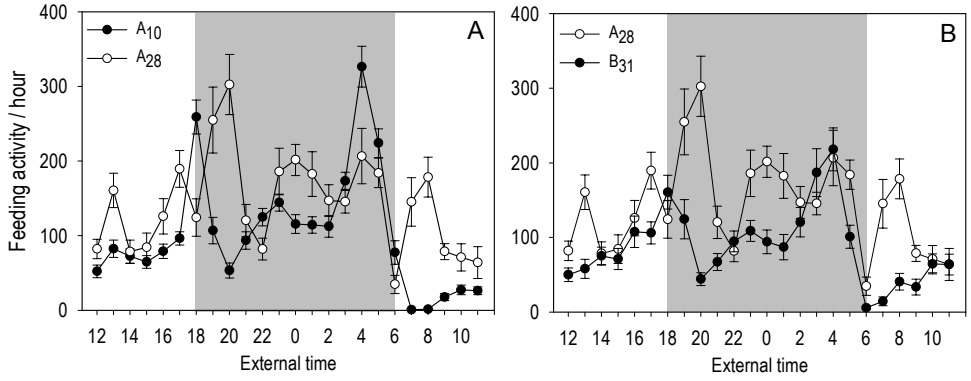

Figure 8. F×BN rats. A. Feeding activity in same rats at young and old age B. Feeding activity in aged rats of 1st and 2nd group

Second (C4) versus first (A10) young group

The A10 group does not show consistent changes compared to the younger C4 group, even though they both display "expected" changes when comparing them to older ones (see Table 2 and 3). The C4 F×BN rats demonstrate more feeding activity than the A10, although feeding was consistently the same when comparing young with aged rats. However, it is not certain that they really ate more, as food intake was not measured. The lower relative daytime feeding activity, and higher amplitude plus ΔQp in the slightly older A10 is difficult to explain. Maybe the circadian system of 4-6 months old rats is not fully "matured" yet. At odds with this is the shorter τ and longer α in the A10 compared to the C4, since this is again in the expected direction for older rats. Possibly, these features of the circadian system age at different speeds. The fact that the even younger Wistar rats (4 months) had much higher amplitudes and ΔQp's (Table 1) could be ascribed to species differences. Wistar rats "age" faster than F×BN rats in many aspects, and F×BN rats are especially known for their long life span and healthy ageing.

Table 3. Circadian feeding behaviour and general activity in F×BN (f1) rats.

2nd group	FEEDING		PIR	
	4-6 months (C_4)	28 months (A_{28})	4-6 months (C_4)	28 months (A_{28})
	n=9	n=9	n=9	n=9
Light-dark cycle (12:12 hrs)				
Daily activity (15 days)	3359 ± 135	3429 ± 248	7493 ± 541***	4547 ± 420
Amp	2.87 ± 0.20	2.72 ± 0.22	2.05 ± 0.08****	1.26 ± 0.03
% Activity in light phase	29.3 ± 1.6**	37.7 ± 1.3	26.1 ± 0.9***	32.5 ± 0.83
Constant darkness (dim red light)				
Daily activity (17 days)	2570 ±114	2688 ± 273	6350 ± 376**	4309 ± 340
τ	24.27 ± 0.04*	23.93 ± 0.17	24.29 ± 0.03*	24.15 ±0.05
ΔQp	93 ± 14	87 ± 37	329 ± 34*	157 ± 23
α	14.9 ± 0.2	14.6 ± 0.3	14.8 ± 0.16*	16.2 ± 0.34
Variation onset (3-h RM)	2.11 ± 0.37	2.74 ± 0.35	1.66 ± 0.23	2.88 ± 0.73
Variation offset (3-h RM)	1.86 ± 0.29*	3.02 ± 0.34	1.67 ± 0.20	2.85 ± 0.62
Variation centre of gravity	2.2 ± 0.26*	2.8 ± 0.29	1.77 ± 0.16	2.35 ± .064

Amp = amplitude (definition: see text), τ = freerunning period, ΔQp = relative strength of strongest significant circadian period, α = activity period, RM = running mean, * p<0.05, ** p<0.01, in italics: n=4.

Second (A28) versus first (B31) aged group

The difference in age between the A28 and the B31 group is only minor (3 months), but from Figure 8B and Table 2 and 3 it is clear that circadian parameters differ (total feeding, α, τ, ΔQp). Taken together, it seems that this particular group (A) has a relatively healthy circadian system, at a late age compared to the B31, but also at a young age, compared to the C4. Some of the divergence between the A28 and the B31 can be explained by the difference in age and experimental conditions, but it is interesting to regard the possibility that the access to a running wheel for most of their adult life (from 7 months onward, with some short periods without) could have had a "conserving" effect on some aspects of the circadian system. Wheel running has been shown to feedback onto the pacemaker (Mrosovsky *et al.*, 1992). The "use it or lose it" theory of Swaab (1991) could be considered here as well. The effect could have been especially pronounced in general locomotor activity (PIR), since this is more close measure to wheel running than feeding, but in the first group, PIR was not measured. It has to be kept in mind as well that the first group consisted of twice as many rats. Finally, the freerunning parameters were calculated in the DD period following an experiment in which the rats had received passive shock avoidance training. Although they were shocked mildly, and this period started more than 10 days after termination of the experiment, a long-lasting effect on behaviour cannot be excluded.

Mus musculus (domesticus)

CBA/ca

CBA/ca mice have a life span of 2-3 years. Here, circadian rhythmicity was studied at the ages of 4 and 17 months. Representative examples of wheel running activity are given in Figure 9. Young mice, placed in DD, usually showed a gradual shortening of their circadian period (τ) . The rhythm seems to become more fragmented during the course of DD, and in some cases mice became almost arrhythmic. Literature on the circadian behaviour of this particular strain was not found. The mice observed here have quite a different circadian pattern compared to other laboratory mouse strains. The freerunning period is rather short in some individuals, with an average τ of 23.1 hrs. This is considerably shorter than other mice strains in DD (e.g. C57bl: τ 23.7; Ola/129: τ 23.8).

Circadian patterning is clearly affected by ageing (Table 4). Decreased activity, a lengthening of τ and α, and lower ΔQp are found. Wheel running is more robust in mice compared to rats, and intense wheel running was still observed older individuals. Aged CBA/ca mice ran about half the distance of their young counterparts. Activity patterns over 24 h cycle in LD are shown in Figure 10. The variability in the onset and centre of gravity did not differ for young and aged individuals, even though a clear decrease in the quantity of running and obvious changes in solidity of the bouts were observed. PIR recordings of general locomotor activity might have given more information on pacemaker changes in this respect.

Remarkable is that for mice, the onset of activity is definitely the most precise parameter, whereas for rats, the offset of activity is generally less variable than the onset (this chapter, and observations in other strains (Wistar and Long Evans). This suggests that mice lock on more to the transition from light to dark (dusk), and rats from dark to light (dawn).

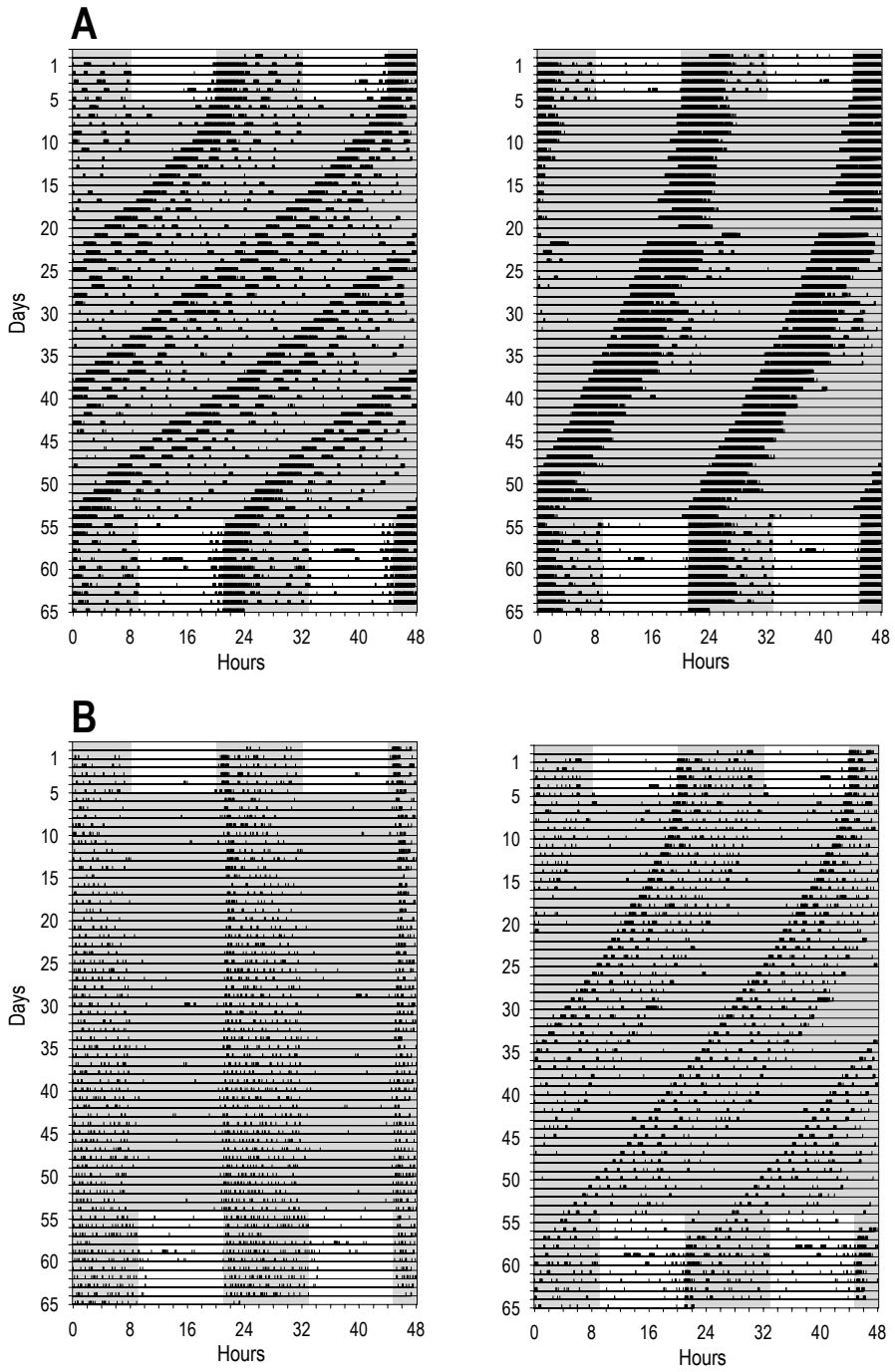

Figure 9. CBA/ca mice: Double-plotted actograms of wheel running activity **A.** 4 months **B.** 17 months.

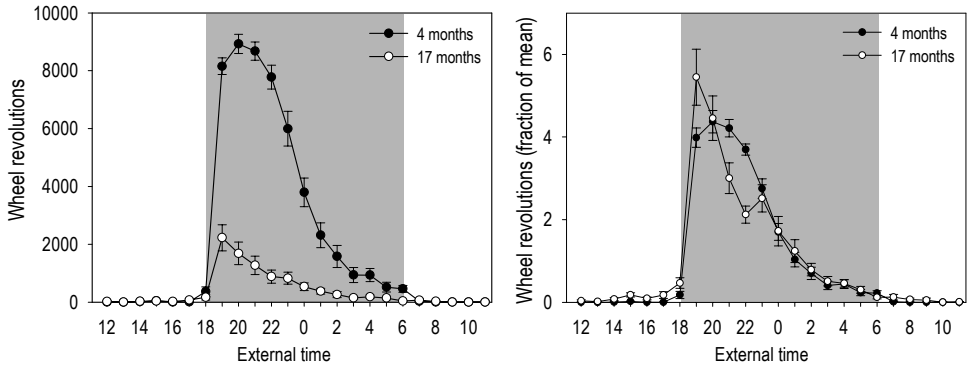

Figure 10. Wheel running activity in CBA/ca mice. Raw data (left panel) and normalised to average daily value (right panel).

Table 4. Circadian wheel running behaviour in CBA/ca house mice

	WHEEL RUNNING	
	4 months	17 months
	n=20	n=20
Light-dark cycle (12:12 hrs)		
Daily activity (10 days)	51300 ± 1817****	9458 ± 1782
Constant darkness (dim red light)		
Daily activity / -τ (13 days)	48306 ± 3679****	8957 ± 2338
τ	23.10 ± 0.07*****	23.69 ± 0.06
ΔQp	2584 ± 148****	1065 ± 81
α	7.74 ± 0.22*	8.47 ± 0.24
Variation onset (3-h RM)	0.55 ± 0.16	0.37 ± 0.09
Variation offset (3-h RM)	1.32 ± 0.13	1.64 ± 0.17
Variation centre of gravity	0.50 ± 0.04*	0.72 ± 0.07

τ = freerunning period, ΔQp = relative strength of strongest significant circadian period, α = activity period, RM = running mean, * p<0.05, **** p<0.0001, ***** p<0.00001.

Central versus peripheral effects of ageing on circadian behaviour

There is not a single parameter best suited to study changes in circadian behaviour with ageing. Experimental conditions, strain, health, and previous history can affect the outcome and should be taken into account. Wheel running is robustly present in older mice, but not in rats. General locomotor activity detection, measured by PIR, is a good way to assess circadian behaviour in aged individuals. This is less dependent on precisely co-ordinated movement. Feeding has proven to be a useful tool to study stability and precision of the pacemaker, since total activity was generally comparable between young and aged individuals, for mice as well as for rats. Calculations are therefore equally powerful across age. The most consistent alterations associated with ageing were decreased locomotor activity, altered day-night distribution (higher percentage daytime feeding), changes in τ (decrease for rats, increase for mice), and larger α.

It is difficult to attribute changes in behaviour to ageing of the pacemaker. Age-related alterations can also be explained by secondary factors, such as motor difficulties. These can readily lead to a decrease in activity levels. The relative contributions of the pacemaker and peripheral processes to the deterioration in overt rhythms can vary: Daan and Oklejewicz (2003) recently demonstrated that the largest variation in precision is due to the latter. In addition, they concluded that phase definitions leading to small variances in cycle length most closely reflect the pacemaker's variance. Using feeding activity for rats -with equal amounts of activity across age-, and wheel running for mice -robustly present in aged mice-, I found considerable differences in centre of gravity (the least variable parameter) between all groups of young and aged animals. I therefore conclude that a significant part of the deterioration of overt rhythms is due to ageing of the pacemaker itself.