The rate of living in tau mutant Syrian hamsters
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Synthesis
The \textit{tau} mutation and non-circadian rhythms

Biological rhythms cover a wide range of frequencies, from milliseconds to years. In this thesis we have shown that an allele described as altering the frequency of the circadian oscillations simultaneously has multiple pleiotropic effects on the frequency of non-circadian biological processes (table 12.1). Within ultradian range of oscillations (periods less than a day) the shortening of circadian period did not have a single predictable effect. The frequency of heart beats was the same for three genotypes of hamsters (chapter 5), confirming the results of Refinetti and Menaker (1993). In contrast, the ultradian feeding cycle was accelerated in homozygote \textit{tau} mutants by 25\% compared to wild-type hamsters (chapter 6). In the ultradian endocrine rhythm in luteinizing hormone (LH) and cortisol Loudon \textit{et al.} (1994) have reported a lower frequency \textit{tau} mutant hamsters.

There is no indication for a single specific brain site involved in the generation of behavioural ultradian rhythms. The arcuate nucleus within the mediobasal hypothalamus is associated with the generation of some endocrine ultradian rhythms (Turek and Van Cauter, 1994; Knobil and Hotchkiss, 1994; Hiruma \textit{et al.}, 1992). Ultradian periodicities may be generated both outside and inside of the suprachiasmatic nucleus (SCN). The multiple unit neural activity (MUA) from SCN in rats exhibits ultradian periods ranged from 3.5 h \textit{in vitro} to about 4 h \textit{in vivo} (Mijer \textit{et al.}, 1997). The ultradian period of feeding cycle and the pulsatile secretion of growth hormone (GH) show a similar circa 4 h period (Richter, 1980). The number of ultradian cycles per 24 h was the same for both feeding and hormone cycles (Tannenbaum and Martin, 1975; 1976; Richter,

\textbf{Table 12.1} The effect of \textit{tau} mutation on biological timing processes.

<table>
<thead>
<tr>
<th>Process</th>
<th>time scale</th>
<th>tau -/- effect</th>
<th>chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/ metabolism</td>
<td></td>
<td>-17%</td>
<td>2,3</td>
</tr>
<tr>
<td>Ultradian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>heart rate</td>
<td>(\approx 150) ms</td>
<td>0%</td>
<td>5</td>
</tr>
<tr>
<td>ultradian hormonal cycle(^1)</td>
<td>(\approx 30) min</td>
<td>+20%</td>
<td>-</td>
</tr>
<tr>
<td>ultradian feeding cycle</td>
<td>(\approx 4) hr</td>
<td>-25%</td>
<td>6</td>
</tr>
<tr>
<td>Circadian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>daily activity cycle</td>
<td>(\approx 1) day</td>
<td>-17%</td>
<td>7</td>
</tr>
<tr>
<td>Infradian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>estrous cycle (^2)</td>
<td>(\approx 4) days</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>torpor cycle</td>
<td>(\approx 6) days</td>
<td>0%</td>
<td>10</td>
</tr>
<tr>
<td>Life span</td>
<td>(\approx 2) years</td>
<td>+15%</td>
<td>11</td>
</tr>
</tbody>
</table>

\(^1\) Loudon \textit{et al.}, 1994; \(^2\) Refinetti and Menaker, 1992a.
1980). It is possible that these two cyclic phenomena are manifestation of the same timing mechanism confined to the SCN. However, SCN lesions in the common vole (*Microtus arvalis*) leave the ultradian rhythm of feeding intact, while lesions extending to the retrochiasmatic area abolish all rhythmicity (Gerkema *et al.*, 1990).

In MUA recordings *in vivo* of tau mutant and wild-type hamsters Yamazaki and co-authors (1998) have shown ultradian frequencies in neural activity of ~1.5 h and ~0.25 h in the SCN which were indistinguishable between the two genotypes. This the ultradian period of MUA in hamsters is dissimilar to that reported for rats and substantially differs from the duration of the feeding cycle (about 4 h) or hormonal secretion (about 0.5 h). This would suggest that the generation of these different ultradian rhythms is not confined to a single ultradian pacemaker in Syrian hamsters and point to multiplicity of generation of the ultradian periodicities.

Long period rhythms are often referred as infradian rhythms. They are often related to periodic reproduction and hibernation. Similar as with ultradian rhythms, the exact role of the circadian system in generation and/or modulation of infradian rhythms is unknown. Female rodents often display a 4-5 days estrus cycle in absence of external time cues (Kent *et al.*, 1968; Alleva *et al.*, 1971). In hamsters, the circadian system controls the production of LH, but the actual ovulation inducing LH surge occurs only every fourth cycle (Fitzgerald and Zucker, 1976; Rusak and Zucker, 1979; Donham and Stetson, 1991). The free running period of LH release hormone in homozygote tau females is about 20 h (Lucas *et al.*, 1999), but estrus takes place not every fourth cycle but every fifth (Refinetti and Menaker, 1992a). The fact that the period of estrous cycle is the same (of approximately 96 h) in hamsters with different circadian periods suggest that estrous and circadian periodicity are generated by different mechanisms, even if they are internally coupled.

Hibernation consists of prolonged torpor bouts with body temperatures approaching the ambient temperature interrupted by periodic euthermy. The function of periodic euthermy and the role of circadian system in triggering arousal and torpor are recurring themes in the hibernation literature (see Strijkstra, 1999; chapter 10, table 10.3). In chapter 10 we have shown that data on arousal-to-arousal frequencies in the three genotype hamsters do not support the hypothesis of a circadian system control over periodicity of torpor and arousal onsets in hibernation.

Of all the rhythms studied only the periodicity of food intake turns out to be affected by the tau allele in a similar manner to the effect on the circadian cycle. This may be a consequence of circadian control over meal timing. It may, however just as well reflect the proportionally increased metabolic rate of tau mutant hamsters.
Circadian rhythmicity in tau mutant hamsters

There are several different properties of the circadian system in tau mutant hamsters on top of the major effect on period length. The shorter circadian period seems to result from shortening of the subjective day - the resting phase of circadian cycle (chapter 3; Osiel et al., 1998; but see Scarbrough and Turek, 1996). The activity phase (α) increases with prolonged time in constant darkness in tau mutants, mostly due to higher fragmentation of an activity pattern. The rhythmicity in tau mutant hamsters has about twice higher variation in the period length and in day-to-day activity onset (precision) compared with wild-types (chapter 7). We have shown that decrease in the rhythm precision can be attributed about equally to a decline in the pacemaker precision and to non-clock peripheral processes.

The characteristics of tau mutant rhythmicity observed at the behavioral level correlate with features of the output system from the suprachiasmatic nucleus (SCN). The neuropeptide arginine-vasopressin (AVP) is a major output system of the SCN. AVP is released in a rhythmic fashion, with high concentration during the subjective day and a drop in level during the subjective night (reviewed in van Esseveldt et al., 2000). In tau mutant hamsters in contrast to wild-types, the content of AVP in the SCN did not differ across the time point measured in the light phase of the circadian cycle (chapter 9). Wild-type hamsters, as most rodent species, release AVP in the SCN in the middle of the subjective day. We did not observe such an oscillation in tau mutants. There was no difference in number of AVP-immunoreactive neurons between genotypes. Consequently, a higher rate of AVP release from tau mutant SCN per 24 h was observed in comparison with wild-type SCN.

It is not clear whether AVP plays a crucial role in generation of rhythmicity but it affects the amplitude of the rhythm, entrainability to light-dark cycle, and period length (Grobblewski et al., 1981; Brown and Nunez, 1989; Murphy et al., 1993; 1996). Additional evidence suggests that AVP may be involved in synchronization of activity of the SCN neurons.

Besides its involvement in modulation of circadian rhythmicity, the AVP level plays a role in memory retrieval processes (Alescio-Lautier and Soumireu Mourat, 1998). The hypothesis has been proposed and currently tested that AVP release from the SCN is crucial for the timing of memory retrieval (B.A.M. Biemans, S. Daan, M.P. Gerkema, E.A. Van der Zee, unpublished observation). As described in rats, the best memory retrieval occurs at multiples of 24 h after the initial learning trial (Holloway and Wansley, 1973a). This oscillation is not present in SCN-lesioned animals (Stephan and
Kovacevic, 1978) and in AVP deficient Brattleboro rats (Biemans et al., in preparation). 

Tau -/- hamsters with a high release level and low circadian amplitude of AVP showed poor overall memory retention as well as no clear oscillation in memory retrieval in the passive shock avoidance paradigm (chapter 8).

In conclusion, the organization on the SCN level in tau mutant hamsters seems to be less precise, possibly due to a lower degree of synchronization between neurons in the SCN and resulting in the low amplitude rhythm of neuropeptide outputs from the SCN. These may have consequences for the expression of overall rhythmicity at the behavioral level.

Metabolism and growth in tau mutant hamsters

The tau mutation has a major impact on metabolism related processes. While shortening the circadian period, it also proportionally enhanced resting and overall metabolic rate per hour, hence it had no effect when expressed per circadian hour (chapter 2). The feeding cycle, which reflects energetic requirements, has been shown to be dependent on genotype (chapter 6). This led to two major hypotheses: (1) the tau allele dictates the overall rate of metabolism through shortening the period of circadian oscillations or (2) the tau allele exhibits independent pleiotropic effects on metabolic processes. To distinguish between these two hypotheses the length of the circadian period was manipulated by deuterium oxide and changes in the metabolic rate were assessed. The ‘heavy water’ administration lengthened the circadian period in a predictable manner but no correlation between the change in a period length and metabolic rate was found (chapter 3). Thus, these results seem consistent with independent pleiotropic effects of the tau allele. However, deuterium administration caused an effect on the circadian period about 30% smaller than tau mutation, and the effect on metabolism might well have been too small to detect. For this reason, I have been interested in seeing whether other genes affecting circadian period would also modify metabolism.

The clock mutation in mice produces in homozygous condition a long (about 28 h) circadian free-running period compared with circa 24 h in wild-types (Vitaterna et al., 1994). An example of wild-type and homozygous clock mouse running wheel activity in constant darkness is presented in figure 12.1. These mice were kindly made available to me by P. Meerlo, J.S. Takahashi, and F.W. Turek (Northwestern University, Chicago). I have measured food intake over three trials, each two days long, and the mean intakes are presented in figure 12.2. Homozygote clock mice had a significantly lower food
intake per 24 h (corrected for body weight), but no difference in the amount of food eaten per a circadian cycle (24 h and 28 h for wild-type and clock -/- mice, respectively). Thus, mice with a longer circadian period had lower energy intake than wild-types but the same per circadian cycle. This indeed supports the notion that the control of the energy demand is partly regulated on endogenous time scale rather than on the 24 h scale.

Hamsters with both alleles mutated were shown to develop smaller body size compared with wild-type hamsters (chapter 2). This effect led to a study on the contributions of mother and pup genotypes to growth in a cross-foster design (chapter 4). It appeared

![Figure 12.1](image1.png)

Figure 12.1 Examples of running wheel activity in constant darkness for a wild-type (A) and a homozygous clock mouse (B).

![Figure 12.2](image2.png)

Figure 12.2 Mass-specific food intake during 24 h (left panel) and during a circadian cycle (right panel) in wild-type and homozygote clock mice. Error bars represent standard deviation.
NOTE: left panel: ***p<.0001; right panel p=.7 (t-test, two tailed)
that both pup and dam genotypes contribute to the variation in growth and body weight. Homozygote tau mutant dams weaned on average smaller pups than wild-type dams. This difference remained present until adulthood. It is possible that body weight differences between genotypes are consequences of (1) different energetic costs of maintenance, (2) discrepancies between the endogenous periods of dam-pup relationship, and/or (3) different timing of ontogeny.

In this context it is of interest that the maternal pacemaker is able to synchronize offsprings very early in gestation: the entrainable circadian pacemaker is present in the fetus of the Syrian hamster two weeks after fertilization (Davis and Gorski, 1988; Reppert and Schwartz, 1986). Viswanathan and Davis (1992) have shown that activity rhythms of heterozygote tau pups were not in phase with rhythm of their wild-type mothers compared with wild-type pups. This means that within one litter of the F2 generation the three genotypes of pups have different phases of their circadian oscillations. Possibly different maternal and offspring synchrony during gestation, when the phase of rhythmicity is set, and later during lactation may be the cue to the maternal genotypic effects on the growth and metabolism of offspring. The effect of the pup’s own genotype on size (chapter 4) may again be considered a pleiotropic effect of the tau allele as distinct from an effect through the modified circadian cycle. Again, we have taken recourse to the clock mutation in mice to see whether there is a relationship between circadian period length and body weight development. We monitored body weight from the age of 50 days onwards in clock -/- mice. These homozygous mutant clock and wild-type mice were produced by crossings of heterozygotes. As seen in figure 12.3, clock -/- mice with a longer circadian period are significantly heavier than wild-type mice. Thus, as in the

![Figure 12.3](image.png)

**Figure 12.3** Body weight development in wild-type and homozygous mutant clock mice.
three genotypes of hamsters, the endogenous circadian period is positively correlated with body size. It is intriguing that the clock and *tau* mutations are changes in complete different elements of the molecular mechanism in rhythm generation (King *et al.* 1997, 2000), yet produce a similar relationship on the length of circadian period and metabolic processes.

Finally, the increased metabolic rate in homozygous *tau* mutant hamsters would lead to the prediction of a lower survival and shorter life span according to the *rate of living* theory. The result is opposite to expectations. Homozygous *tau* mutant hamsters of both sexes lived longer than wild-types (chapter 11). This result is highly intriguing. It demonstrates that the mutation causing a severe maladaptation in behavior and physiology, which would surely be rapidly weeded out by the natural selection in the hamster real world, may yet enhance viability in a constant environment. We do not know why this is the case. Possibly the leanness of the mutant’s body, itself a consequence of its higher mass-specific metabolism resulting from the reduced rest part of the circadian cycle, renders it fitter to survive into old age, an age probably rarely achieved in nature.

In summary, this thesis has demonstrated that the *tau* mutation selectively accelerates the circadian cycle and no other cyclic temporal patterns, except for the ultradian feeding cycle. The feeding cycle is probably adjusted to the metabolic rate that is enhanced in *tau* mutant hamsters, most likely due to selective reduction of the rest phase of the circadian cycle. The enhanced metabolism had an expected effect of reductions in growth, adult body size and adult mortality in homozygous *tau* mutants compared to their wild-type siblings. So in the end, the circadian system is linked to some aspects of the rate of living, albeit in complex, unanticipated ways.