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Report

Lack of Food Anticipation in *Per2* Mutant Mice

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Summary

Predicting time of food availability is key for survival in most animals. Under restricted feeding conditions, this prediction is manifested in anticipatory bouts of locomotor activity and body temperature. This process seems to be driven by a food-entrainable oscillator independent of the main, light-entrainable clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus [1, 2]. Although the SCN clockwork involves self-sustaining transcriptional and translational feedback loops based on rhythmic expression of mRNA and proteins of clock genes [3, 4], the molecular mechanisms responsible for food anticipation are not well understood. *Period* genes *Per1* and *Per2* are crucial for the SCN's resetting to light [5–7]. Here, we investigated the role of these genes in circadian anticipatory behavior by studying rest-activity and body-temperature rhythms of *Per1* and *Per2* mutant mice under restricted feeding conditions. We also monitored expression of clock genes in the SCN and peripheral tissues. Whereas wild-type and *Per1* mutant mice expressed regular food-anticipatory activity, *Per2* mutant mice did not show food anticipation. In peripheral tissues, however, phase shifts of clock-gene expression in response to timed food restriction were comparable in all genotypes. In conclusion, a mutation in *Per2* abolishes anticipation of mealtime, without interfering with peripheral synchronization by feeding cycles.

Results

To determine whether *Per1* and *Per2* genes are involved in the adaptation to restricted feeding conditions, we exposed wild-type (WT), *Per1*, and *Per2* mutant mice either to a hypocaloric feeding (HF) schedule or to temporally restricted (TR) food access. No difference was found in the amount of wheel-running activity among the three genotypes fed ad libitum (AL) in light-dark (LD) conditions ($p = 0.51$). Daytime temporal food restriction is a potent synchronizer of peripheral clocks in nocturnal rodents held under light-dark conditions and does not alter clock-gene expression in their SCN [8–11]. However, HF can cause significant phase advances of circadian rhythms of locomotor activity and melatonin [12, 13] as well as alterations of both SCN clockwork and circadian responses to light [14]. As previously shown [13–15], WT mice under both TR and HF conditions showed a bout of wheel-running activity before feeding time that occurred at Zeitgeber time (ZT) 4 (3592 ± 1001 and 2208 ± 505 wheel revolutions, respectively; Figure 1A; see also Figure S1A in the Supplemental Data available online); we will refer to this activity as food-anticipatory activity (FAA). FAA is defined as the total number of activity bouts that occur during the 2 hr immediately preceding the daily mealtime. Statistical analysis revealed no difference between TR and HF conditions ($p = 0.67$, not significant [NS]) but a significant difference between AL and both TR and HF conditions ($p < 0.05$) for WT mice. *Per1* mutant mice displayed FAA comparable to that of WT animals (2108 ± 650 and 3208 ± 779 wheel revolutions in TR and HF, respectively; NS; Figure 1B and Figure S1B). No statistical difference could be found when WT and *Per1* mutant mice were compared in the same feeding conditions (AL: $p = 0.98$; TR: $p = 0.52$; and HF: $p = 0.70$). Interestingly, *Per2* mutant mice did not show significant FAA (482 ± 171 and 166 ± 53 wheel revolutions in TR and HF, respectively; $p < 0.05$ compared to WT mice; Figure 1C and Figure S1C). Moreover, no difference was found in wheel-running activity in different feeding conditions for the *Per2* mutants (AL versus TR: $p = 0.50$; AL versus HF: $p = 0.50$; and TR versus HF: $p = 0.9$). Because FAA is supposed to reflect an output of a food-entrainable oscillator (FEO), FAA can be expected to be present during fasting [1, 2]. To avoid masking by light, we tested food anticipation under fasting conditions in constant darkness (DD). Both WT and *Per1* mutant mice displayed FAA under these conditions (arrows in Figures 1A and 1B; see also Figures S1A and S1B). In contrast, fasted *Per2* mutant mice did not show any similar activity bout (Figure 1C and Figure S1C). This supports the finding that lack of activity before feeding time in light-dark conditions corresponds to lack of FAA, indicating that *Per2* plays a critical role in the process of food anticipation. However, a possible reappearance of FAA in *Per2* mutant mice during fasting might have been masked by the intrinsic free-running-activity rhythm (but see below).

To assess the influence of food restriction on the SCN, we released mice after TR or HF conditions into DD with food provided AL. In accordance with previous findings

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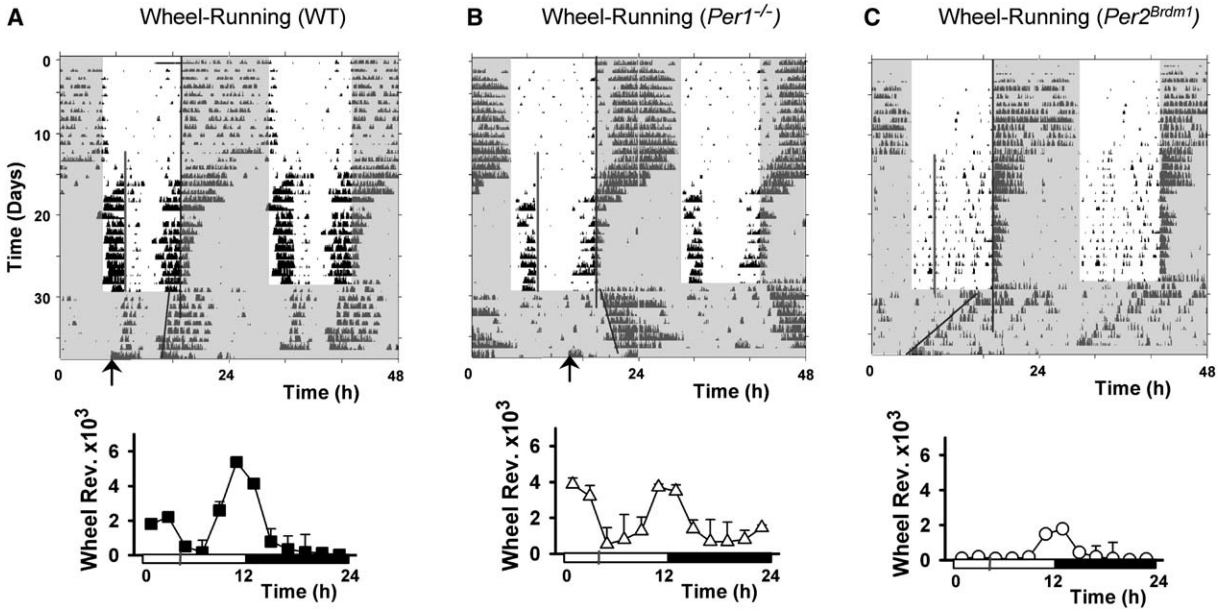

Figure 1. Daily Wheel-Running Activity

Wild-type (A), *Per1* (B), and *Per2* (C) mutant mice with hypocaloric feeding under light-dark conditions. Activity is plotted as an actogram with each horizontal line corresponding to two consecutive days and with the second day being double plotted on the next line. Gray shading indicates lights off. Animals were fed ad libitum, submitted to hypocaloric feeding, and subsequently released into constant darkness with food ad libitum with the exception of the last day (arrow) when no food was accessible. The gray line indicates the time when hypocaloric food was provided. The arrow shows food-anticipatory activity at the expected time in both wild-type and *Per1* mutant mice fed ad libitum in constant darkness. The bottom graph represents the mean daily-activity profile during the last 8 days of hypocaloric feeding period (n = 6 in WT and $Per1^{-/-}$ mice and n = 4 in $Per2^{Brdm1}$ mice; mean ± SEM). The gray line on the X axis indicates time of feeding. Note the lack of food-anticipatory activity in *Per2* mutant mice (C).

[8–10], temporal food restriction did not induce significant phase shifts (−18 ± 24 min) in the locomotor output in WT mice (Figure S2B). In contrast, HF led to phase advances of 82 ± 18 min. Interestingly, *Per1* mutant animals displayed phase delays under temporal food restriction (−71 ± 25 min), whereas under HF, no average phase change was observed (−15 ± 32 min). Furthermore, *Per2* mutant mice exhibited large phase advances under both restricted feeding conditions (TR: 153 ± 26 min and HF: 199 ± 72 min). Note that no phase shifts are observed in control *Per1* and *Per2* mutant mice that were fed AL and transferred from the same light-dark conditions to DD [7, 16, 17].

To assess whether the lack of wheel-running anticipation in *Per2* mutant mice is due to a light-masking effect that would directly suppress wheel-running activity in nocturnal mice, we investigated general cage activity and body temperature in WT and *Per2* mutant mice exposed to HF in constant light (LL; Figures 2A–2F) and DD (Figures S3A–S3F). WT mice under both conditions showed FAA during the 2 hr before feeding time, indicated by the shaded area in Figure 2E and Figure S3E. This FAA was not observed in *Per2* mutant mice (LL: 5.6 ± 1.5 versus 1.9 ± 0.7 a.u., $p < 0.05$ and DD: 6.9 ± 1.7 versus 2.3 ± 0.2 a.u., $p < 0.05$; sum in the shaded area of Figure 2E and Figure S3E). The daily amount of total activity was not significantly different in WT compared to *Per2* mutant mice either in LL (18.3 ± 5.6 versus 13.6 ± 4.3 a.u., respectively; NS) or in DD (26.1 ± 9.2 versus 24.0 ± 9.4 a.u., respectively; NS). Note that under both LL and DD conditions, the locomotor activity appeared to be synchronized to mealtime in WT mice, whereas in *Per2* mutant mice, this rhythm seems to free-run, and no FAA can be observed (Figure 2A and 2C; see also Figures S3A and S3C). Under both LL and DD conditions, WT mice showed a daily increase of body temperature during the 2 hr before feeding time, concomitant with FAA, indicated by the shaded area in Figure 2F and Figure S3F. There was also a daily 2 hr postprandial increase of body temperature, corresponding to the so-called diet-induced thermogenesis (DIT). Compared to that in WT mice, body temperature in *Per2* mutant mice was significantly reduced during the 2 hr before mealtime (LL: 36.2 ± 0.4°C versus 34.6 ± 0.3°C, $p < 0.05$; DD: 37.0 ± 0.4°C versus 35.5 ± 0.5°C, $p < 0.05$; average in the shaded area of Figure 2F and Figure S3F). By contrast, DIT was similar between WT and *Per2* mutant mice (LL: +1.7 ± 0.4°C versus +2.6 ± 0.4°C, NS; DD: +1.0 ± 0.5°C versus +1.9 ± 0.5°C, NS; respectively). Similar to the activity rhythms, temperature rhythms appear to be synchronized to mealtime in WT mice, whereas in *Per2* mutants, this rhythm free-runs (Figures 2B and 2D; see also Figures S3B and S3D). Hence, peaks before mealtime every 10–15 days do not correspond to an anticipatory bout of body temperature but rather represent the free-running-activity rhythm. However, it is of note that restricted feeding maintains circadian rhythmicity in the *Per2* mutant mice housed in constant darkness. After the last day of hypocaloric feeding in DD, food was provided AL with a 6 hr delay compared to previous mealtime (horizontal white arrow on Figures S3A–S3D). WT mice displayed both an anticipatory increase in body temperature and a delayed DIT (Figure S3B). By contrast, *Per2* mutants showed only