

Research report

Food-entrained circadian rhythms in rats are insensitive to deuterium oxide

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Abstract

Rats anticipate a scheduled daily meal by entrainment of a circadian pacemaker separate from the light-entrainable circadian pacemaker located in the suprachiasmatic nuclei (SCN). The site and molecular mechanisms of the food-entrainable pacemaker are unknown. The intrinsic period (τ) of the SCN pacemaker is significantly lengthened by deuteriation. Sensitivity of food-entrained circadian rhythms to D₂O (25% in drinking water) was evaluated in intact and SCN-ablated rats entrained to daily feeding schedules. In intact rats fed ad-libitum, D₂O lengthened τ sufficiently to drive activity rhythms out of entrainment to the light–dark cycle. By contrast, food-entrained rhythms were surprisingly resistant to modulation by D₂O. The mean daily onset time of food anticipatory activity in rats with complete SCN-ablations was not affected by up to 28 days of D₂O intake. Transient delays and disruption of anticipatory activity were evident in intact and one partial SCN-ablated rat during D₂O treatment, but these are interpretable as effects of coupling and/or masking interactions between a D₂O-sensitive light-entrainable pacemaker, and a D₂O-resistant food-entrained pacemaker. Differential sensitivity to D₂O suggests diversity in the molecular mechanisms of food- and light-entrainable circadian pacemakers in mammals. D₂O may have utility as a screening test to identify putative food-entrainable pacemakers from among those central and peripheral tissues that can express circadian oscillations of clock genes independent of the SCN. © 2001 Elsevier Science B.V. All rights reserved.

Theme: Neural basis of behavior*Topic:* Biological rhythms and sleep*Keywords:* Circadian rhythm; Deuterium oxide; Food entrainment; Wheel running; Clock gene**1. Introduction**

Circadian rhythms in mammals are mediated by a multioscillator system (for reviews, see [12,15]). A master circadian pacemaker resides in the hypothalamic suprachiasmatic nucleus (SCN). Output signals from this pacemaker drive the daily rhythms in behavior and physiology that are normally entrained to the daily light–dark (LD) cycle, but persist (‘free-run’) in constant light (LL) or dark (DD). Circadian rhythms in many behavioral and physio-

logical variables can also be entrained by daily schedules of restricted food access [14]. However, the circadian pacemaker generating these rhythms is not dependent on the SCN. This has been established by a thorough analysis of the properties of daily rhythms of food-anticipatory activity (FAA) and physiology (e.g., plasma corticosterone) that are expressed by SCN-ablated rats maintained on one or two daily meals provided at a fixed time of day. These rhythms exhibit canonical properties of an entrained, circadian oscillatory process, including circadian limits-to-entrainment (i.e., FAA occurs only if feeding intervals are within the circadian range; [19]), gradual resetting in response to shifts of the presumed entraining stimulus (i.e., mealtime; [20,21]), and persistence (‘free-run’) during total food deprivation or at the entrainment limits [6,18,20,21].

Despite over 20 years of research, the physical substrate of the food-entrainable circadian pacemaker has eluded

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localization (reviewed in [14]). In that time, much has been learned about the molecular mechanisms of the light-entrainable circadian clock in the SCN. This clock is now known to consist of interlocking negative and positive feedback loops between several clock genes and their protein products [17]. These genes are also expressed in fibroblast cell lines and peripheral tissues outside of the SCN, where expression can be induced to oscillate for at least a few circadian cycles by application of an appropriate stimulus (e.g., serum shock; [1,3,4,8,24–26]). Furthermore, in vivo, circadian expression of *Per* genes in liver cells but not SCN cells is entrainable by restricted feeding schedules [8,22]. These findings provide direct support for a distributed, multioscillator model of the mammalian circadian system, and suggest candidate substrates for its food-entrainable component. Moreover, the induction of clock gene cycling by stimuli applied to tissue culture may be a cellular homologue for the induction of circadian behavioral rhythms by scheduled feeding in previously arrhythmic, SCN-ablated rats.

If the food-entrainable pacemaker is comprised of the same set of clock genes and feedback loops responsible for SCN clock function, then this pacemaker should be susceptible to gene mutations and biochemical treatments known to alter cycling of the SCN pacemaker. One treatment that has a potent effect on SCN-dependent rhythms is deuterium oxide (D_2O). Deuteration robustly lengthens the circadian period (τ) expressed in constant conditions and phase delays or disrupts entrainment to LD cycles in all uni- and multicellular organisms studied to date, e.g. [7,9–11,13,16,23]. Here we report an exception; D_2O has little effect on food-entrained circadian activity rhythms in rats.

2. Methods

2.1. Subjects and apparatus

Adult, male Sprague Dawley rats (Charles River, PQ) were housed individually in Wahmann activity cages in ventilated, light-controlled isolation cabinets. The cages were equipped with running wheels (35 cm diameter) and automated feeders. Food was provided in a cup accessible through a window in the door of the side cage. Wheel running activity was detected by microswitches. In some cases (SCN Group 2, see below), food-bin activity was detected by interruption of a photobeam aligned over the food cup. Activity devices were monitored continuously by computer, and activity counts were summed and stored to disc at 10-min intervals for analyses off-line.

2.2. Procedures

Four groups were tested. The first group ($N=2$) was used to confirm that D_2O at a concentration of 25% in drinking water can disrupt LD entrainment. The rats were

maintained in LD 12:12 (~15 lux at cage level) with tap water for 2 weeks, 25% D_2O in place of tap water for 15 days, and tap water again for a final 2 weeks. Food was available ad-libitum.

The second group was used to assess the suitability of intact rats for evaluating effects of D_2O on food-entrained rhythms. If D_2O lengthens τ of the food-entrainable pacemaker, then its phase of entrainment to a feeding cycle, marked by the time at which FAA begins relative to scheduled mealtime, should be delayed. If the change in τ is sufficiently large, then the pacemaker may be driven from stable entrainment to a state of oscillatory free-run. Rats ($N=3$) were maintained under LD for 2 weeks with free access to food and water. Food was then restricted to 3 h each day beginning 3 h after lights-on. After 25 days, LD was replaced by LL (5–10 lux). After another 25 days, water was replaced by 25% D_2O for 18 days. Food restriction continued for a final 7 days after withdrawal of D_2O .

Two groups were used to evaluate the effects of D_2O on food-entrained rhythms in rats with SCN-ablations. Eight rats (SCN Group 1) were subjected to radiofrequency ablations aimed at the SCN and allowed to recover with free access to food for 5 weeks in LD, and 2 weeks in LL (~5 lux). Food was then restricted to 3 h each day for 9 weeks. Five rats received 25% D_2O in place of water during the last 25–28 days of restricted feeding, while the other three received tap water.

An additional 12 rats (SCN Group 2) received radiofrequency ablations aimed at the SCN. After 3 weeks in LL, food was restricted to 3 h each day for 7 weeks. During the last 2 weeks of restricted feeding, six of the rats received 25% D_2O as drinking water. All rats were then food deprived for 75 h, at which time the D_2O regime was terminated and food and regular water were provided ad-libitum.

2.3. Surgery and histology

Prior to behavioral tests, rats were anesthetized with sodium pentobarbital and positioned in a Kopf rodent stereotaxic device. SCN lesions were created by radiofrequency current passed through insulated '0' insect pins, placed at 0.2–0.3 mm posterior, ± 0.3 mm lateral and 9 mm ventral to the skull at bregma, with the mouthpiece 3.3 mm below the interaural line. After behavioral tests were complete, SCN-ablated rats received an overdose of sodium pentobarbital and were perfused through the heart with saline and formalin. The brains were removed, post-fixed in glucose-formalin, frozen-sectioned at 30 μ m intervals, mounted on slides and stained with cresyl violet to quantify the extent of SCN damage.

2.4. Data analysis

Visual inspection of actograms and average waveforms indicated that FAA was concentrated within 3 h of

mealtime. FAA was quantified by averaging data in 1 week blocks, and recording the time at which activity exceeded the 24 h mean for the block (excluding activity during mealtime). This point was defined as the onset of FAA, and was expressed as FAA duration by conversion to minutes in advance of mealtime. FAA duration before and during D₂O treatment was compared using paired and independent *t*-tests as appropriate. Group means in the text are reported $\pm$ standard deviation.

3. Results

3.1. Intact rats: LD-entrained rhythms

D₂O at a concentration of 25% in drinking water had a potent effect on circadian wheel running rhythms of intact rats entrained to a standard LD cycle (Fig. 1). Prior to deuteration, nocturnal activity onset occurred within 30 min of dark onset. Activity onset was delayed by $\sim$ 1 h within 48 h of D₂O treatment and by $\sim$ 4 h within 1 week, and lagged a full 24 h by the end of the second week. After

D₂O treatment, one rat continued to ‘free-run’ for another week before reentraining to LD (Fig. 1b), while the second rat showed an extended after-effect in the form of a strongly negative phase angle of entrainment (Fig. 1a).

3.2. Intact rats: food-entrained rhythms

During restricted feeding in LD, the three intact rats showed 1 or 2 bouts of wheel running early in the night, and robust FAA beginning 3–4 h prior to mealtime in the day (Fig. 2a–c). In LL, nocturnal activity bouts began a clear free-run in one case (Fig. 2a), with $\tau > 24$ h. In the other two cases, activity levels outside of the FAA bout were lower, and a free-run was more difficult to track. However, τ appeared close to 24 h in one case (Fig. 2b), and greater than 24 h in the other (Fig. 2c). In all three cases, the onset of FAA progressively delayed during the first 3–8 days of LL, but then advanced. In one case (Fig. 2a), the onset of FAA was strongly influenced by the phase of the free-running component of activity.

During D₂O treatment, all three rats showed a prominent component of activity that progressively delayed with

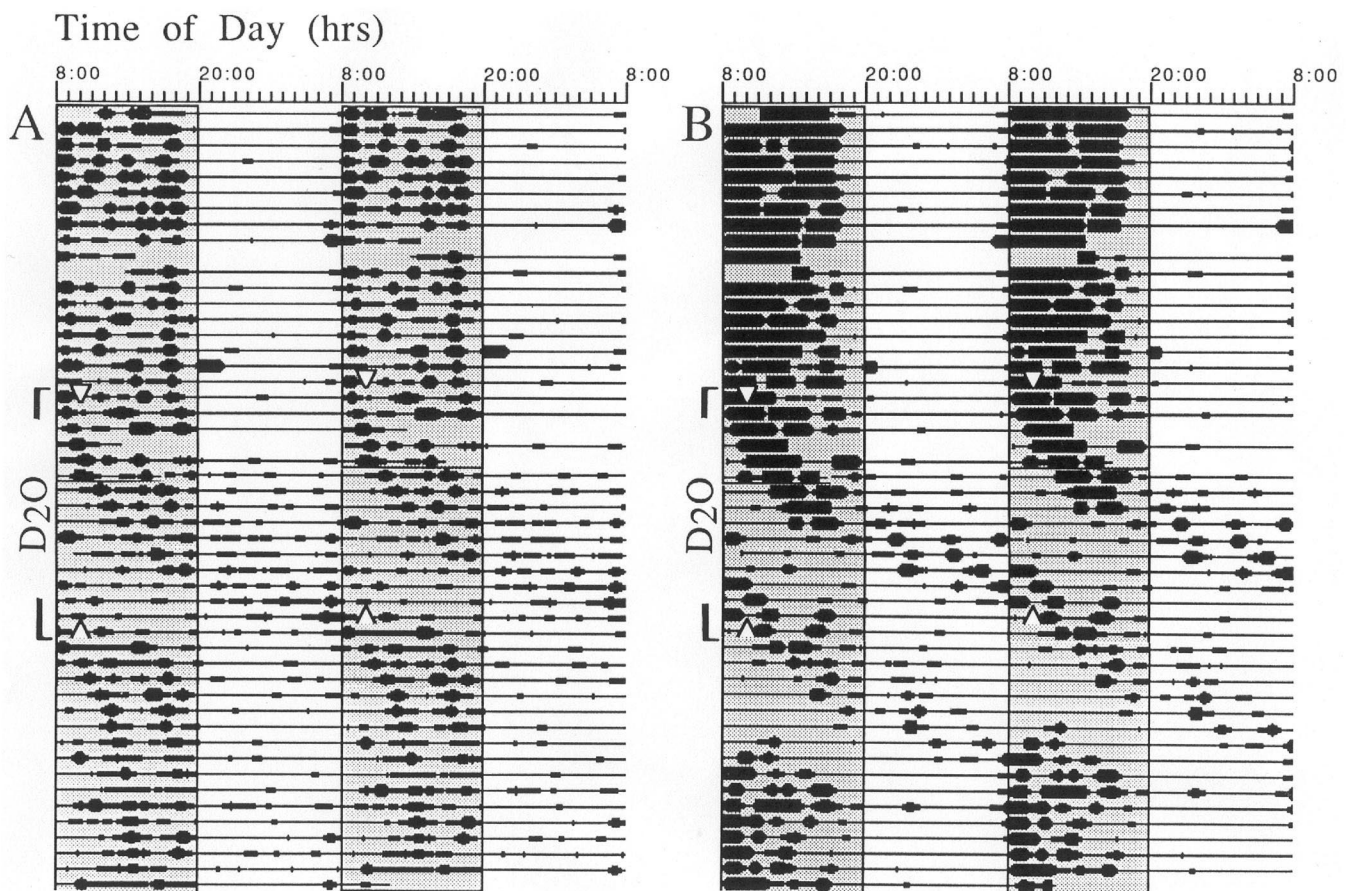


Fig. 1. Double-plotted actogram of wheel running activity from two intact rats maintained under a 12:12 LD cycle with free access to food. Each line represents two consecutive days, with time in 10 min bins plotted left-to-right. Consecutive days are also aligned vertically. Time bins during which activity occurred are indicated by a vertical deflection from the zero activity horizontal line. Days of D₂O treatment are indicated by the brackets and the triangles. To increase clarity, data were smoothed once using a weighted (1:4:1) running average. The dark period is indicated by shading. Blanks represent missing data due to equipment failure.

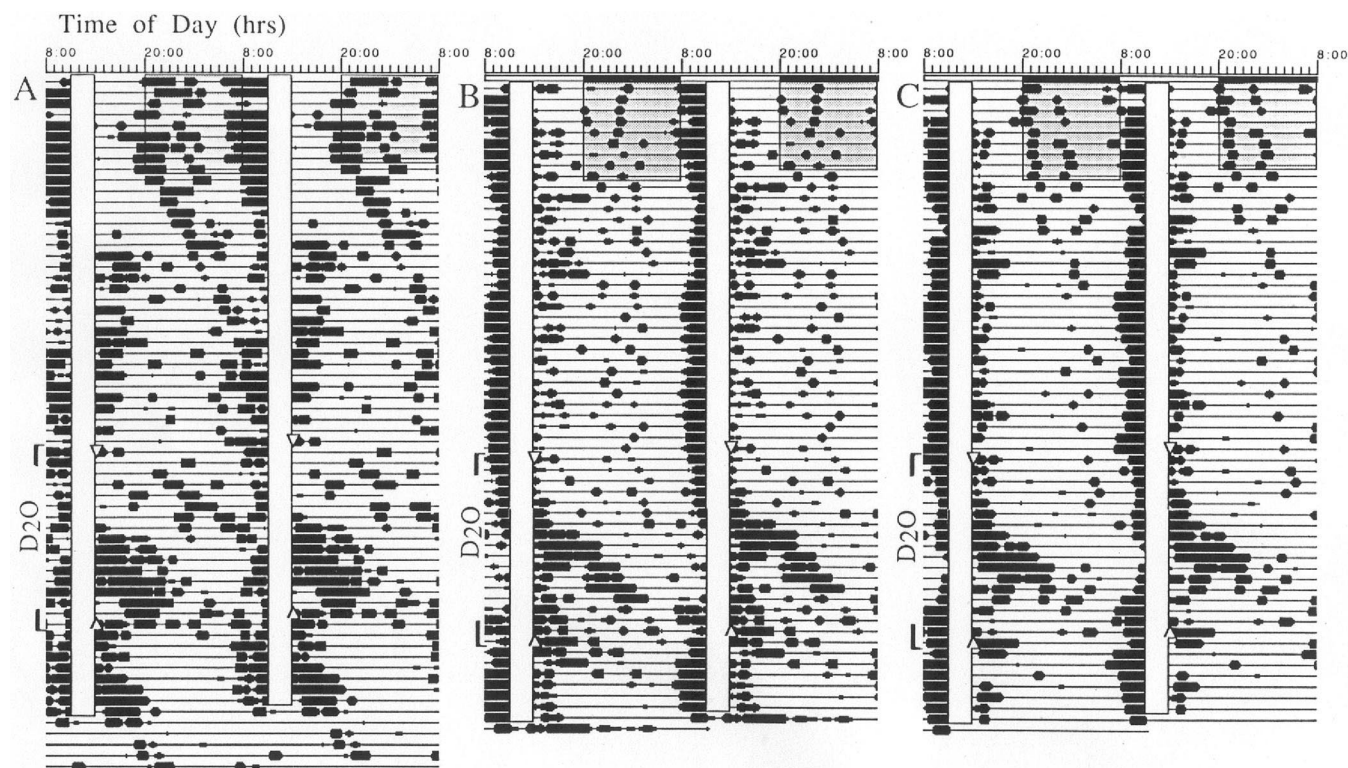


Fig. 2. Double-plotted actograms of wheel running from three intact rats on a restricted feeding schedule in LD and LL. Mealtime is indicated by the opaque vertical bar. See Fig. 1 for other conventions.

respect to mealtime. The onset of FAA delayed in parallel (Fig. 2b,c), but then advanced during the last few days of treatment. This change in the timing of FAA may reflect interactions with free-running, light-entrainable rhythms that are lengthened by D_2O . The interpretive complexity introduced by the presence of rhythms mediated by the D_2O -sensitive SCN prompted us to conduct further studies using only SCN-ablated rats.

3.3. SCN-ablated rats Group 1: food-entrained rhythms

Three of eight rats that received lesions directed at the SCN were maintained on tap water throughout ad-libitum and restricted food access. Of these, one was arrhythmic in LL prior to restricted feeding (Figs. 3d, 4d), and two exhibited a low amplitude wheel running rhythm. During restricted feeding all three rats exhibited robust FAA that was stable throughout the 9 week schedule (e.g., Figs. 3d, 4d). Post mortem inspection of brain sections revealed sparing of ~15% of SCN in each case. Minor damage to anterior and periventricular hypothalamus adjacent to the SCN was also evident.

The other five rats with SCN lesions received D_2O during the last 4 weeks of the restricted feeding schedule. Four of these rats were arrhythmic prior to restricted feeding and showed a robust FAA during the feeding schedule that was unaffected by D_2O treatment (e.g. Figs.

3a–c, 4a–c). Post mortem inspection revealed complete lesions in three cases, and sparing of ~10% of the SCN in one case. The remaining rat exhibited a weak free-running rhythm prior to restricted feeding. FAA onset in this animal exhibited a transient delay during the second and third weeks of D_2O treatment, similar to that exhibited by intact rats during D_2O treatment. This rat was found to have retained ~20% of one SCN intact.

3.4. SCN-ablated rats Group 2: food-entrained rhythms

Both wheel running and food-bin activity were monitored in this group. All 12 rats were arrhythmic in both activity measures in LL prior to restricted feeding and were found on histological analysis to have sustained complete SCN ablations. During the first 5 weeks of restricted feeding, 10 of 12 rats exhibited robust FAA in food-bin approaches, while two showed little or no FAA on most days. Data from these two rats were therefore excluded from tests of group means.

D_2O treatment during weeks 6 and 7 of the feeding schedule had no effect on FAA duration in the food-bin measure (e.g. Fig. 5b); the group mean average waveforms for the last week of water and the 2 weeks of deuteriation are virtually identical (Fig. 6a). Activity appeared to be elevated following mealtime during the second week of D_2O treatment, but this feature of the group waveform is

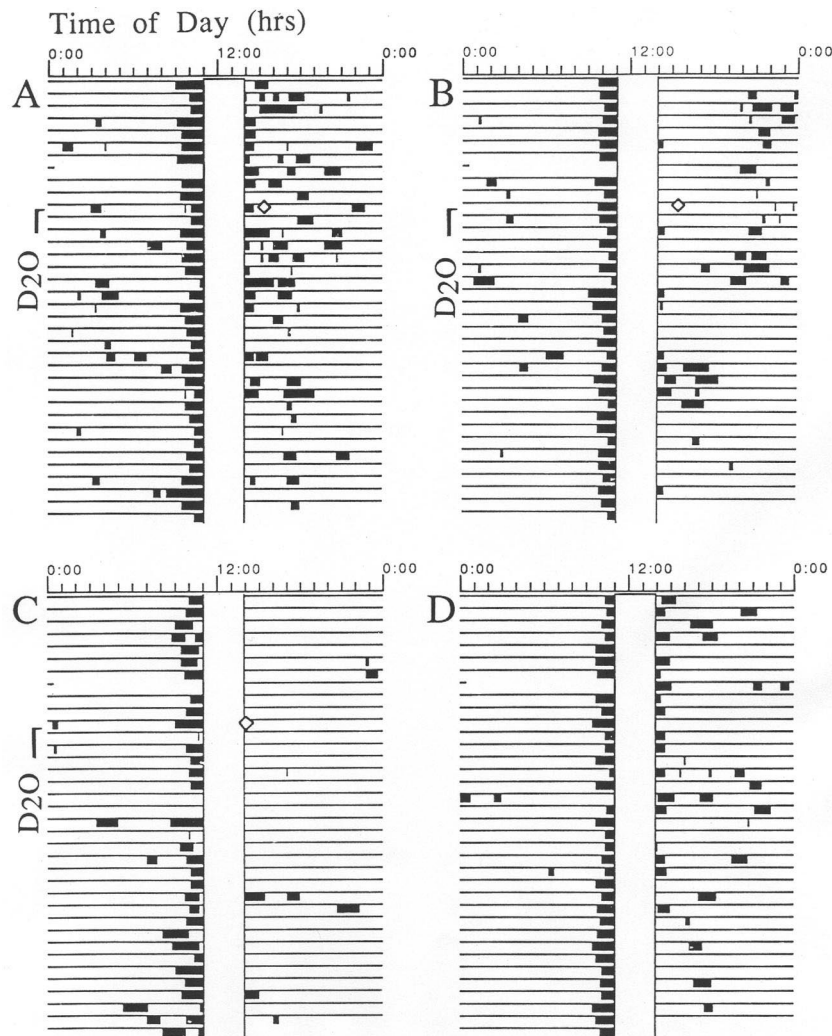


Fig. 3. Single-plotted actograms of running activity from three rats with complete SCN ablations (A–C), and one rat with a partial SCN ablation (D), but which lacked circadian rhythms during ad-lib feeding prior to restricted feeding. In panels A–C, the onset of D₂O treatment is indicated by the diamond and the brackets. See Fig. 1 for other conventions.

representative of only one animal. FAA duration averaged 130 ± 25 min prior to deuteration, and 124 ± 26 min during the last week of D₂O ($t=0.43$, $P>0.1$). SCN-ablated rats maintained on tap water also did not show a significant change in FAA duration across these weeks (Figs. 5a, 6b; 132 ± 28 min and 144 ± 38 min, respectively; $t=0.8$, $P>0.1$). FAA duration did not differ between groups on the last week of D₂O treatment (independent $t=1.83$, $P=0.09$).

Total daily wheel running averaged only 100–300 revolutions/day in the SCN-ablated rats that received D₂O, but most of this running occurred within 3 h of meal onset and D₂O did not affect its timing. Wheel running data were pooled with that from rats in Group 1 that were also arrhythmic prior to restricted feeding. Mean FAA duration in this combined group of nine was 142 ± 27 min during the week prior to D₂O treatment, and 126 ± 25 min during week 2 of D₂O treatment ($t=1.16$, $P>0.1$).

SCN Group 2 rats were food deprived for 75 h following the last meal of the daily feeding schedule. All of the rats exhibited at least some activity during the expected feeding time (e.g. Fig. 5a,b), but the magnitude of this response was weak in most cases after the first 24 h, which precluded a meaningful assessment of its timing. Control and D₂O-treated rats did not differ in this respect.

3.5. D₂O intake

Intact and SCN-ablated rats subjected to restricted feeding consumed similar amounts of D₂O (36.3 ± 4.8 ml/day and 41.2 ± 7.3 ml/day, respectively; $t=2.72$, $P>0.1$). The two intact rats maintained on ad-lib food intake averaged 48.2 ± 5.3 ml/day of D₂O, a larger amount consistent with their greater body weight (they were not food deprived).

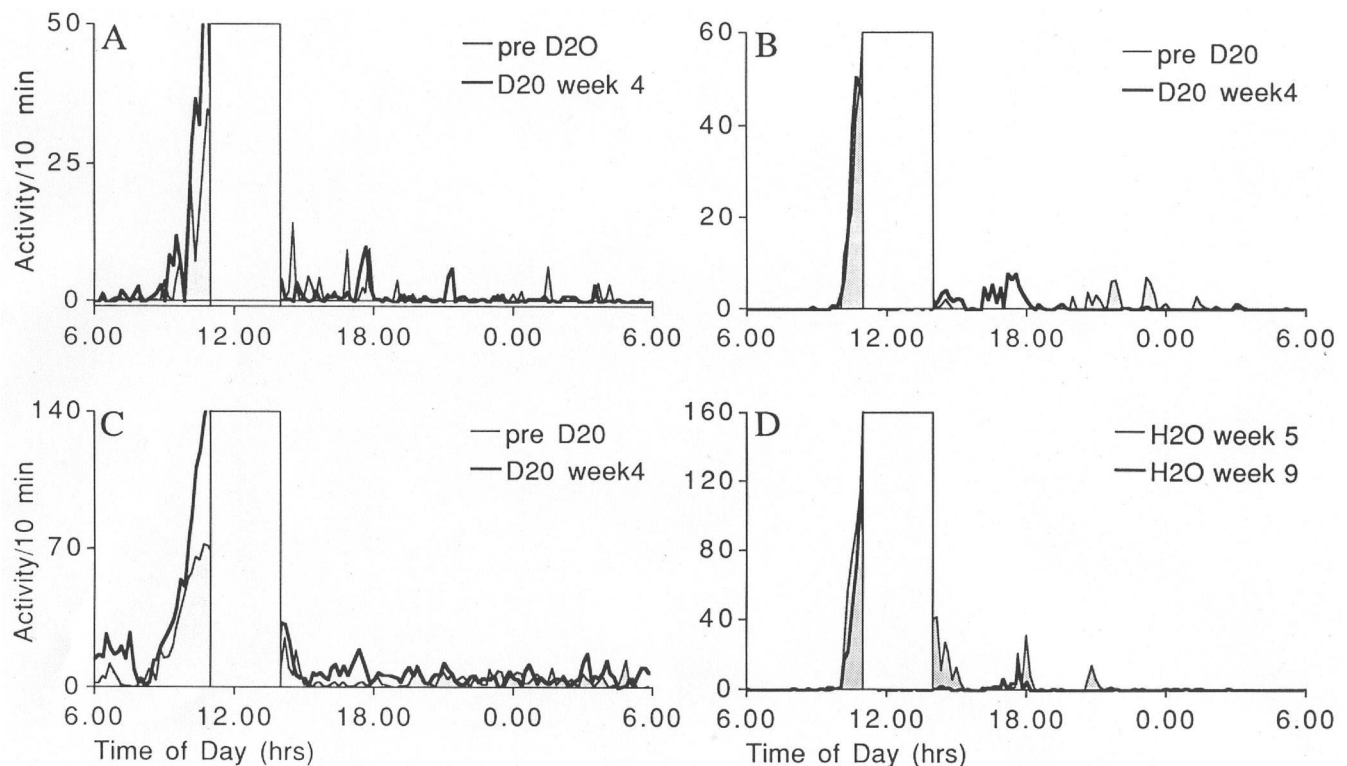


Fig. 4. Average waveforms of food-bin activity from three rats with complete SCN ablations (A–C), and one rat with a partial SCN ablation (D). In panels A–C, the thin line and stippling represent activity during the last week of water access prior to D₂O, and the heavy line represents activity during the last week of D₂O. The vertical bar represents mealtime.

4. Discussion

Previous work has demonstrated that τ of free-running rhythms in rodents fed ad-libitum is lengthened by 3–7% during chronic treatment with 20–25% D₂O, e.g., [7,9,13,16,23]. In the present study, 25% D₂O lengthened τ sufficiently to rapidly drive circadian activity rhythms out of entrainment with the LD cycle. Similarly robust effects have been observed previously, e.g., in one study, LD entrainment failed in five of 11 mice at D₂O concentrations of 20–30%, and was phase delayed by 5–6 h in the other mice [9].

Given the robustness of these effects, the major result of this study was unexpected; food-entrained rhythms in SCN-ablated rats were resistant to modulation by D₂O. In the nine SCN-ablated rats from Groups 1 and 2 that lacked free-running rhythms in LL but exhibited FAA during restricted feeding, the onset of FAA relative to mealtime was virtually unaltered by 2–4 weeks of D₂O treatment.

Data from rats with intact or partially intact SCN are more difficult to interpret. These rats showed clear delays of FAA during the first 7–10 days of D₂O intake, which is the expected result if D₂O lengthens τ of the food-entrainable pacemaker. However, FAA duration recovered during the last week of treatment. Moreover, a similar, albeit less dramatic delay of FAA onset was evident in the three

intact rats during the first week of LL prior to D₂O treatment. This suggests that modulations of FAA duration may have been mediated indirectly by interactions between the food-entrainable pacemaker and a D₂O-sensitive light-entrainable pacemaker, rather than by a direct action of D₂O on the former. FAA may initially delay by virtue of coupling forces exerted on the food-entrainable pacemaker by the light-entrainable pacemaker, which is delayed (slowed) by both LL and D₂O. Subsequent recovery of FAA duration could reflect loss of coupling, possibly due to increasing disparity between τ of the two pacemakers. Some of the reduction in the duration of FAA early in D₂O treatment could also reflect masking effects of the light-entrainable pacemaker. As this pacemaker free-runs through mealtime, the rest phase of its cycle would occur during the hours preceding mealtime, which may oppose the expression of FAA. The results from the intact rats, while intriguing, are inconclusive with respect to the sensitivity of the food-entrainable pacemaker to D₂O.

In the experiments reported here, the effects of D₂O were assessed in rats that were stably entrained to feeding schedules, rather than free-running in constant conditions. In principle, the phase angle of entrainment could remain stable despite changes in pacemaker τ if the zeitgeber (entrainment cue) is of sufficient strength. This raises the possibility that D₂O may affect τ of the food-entrainable

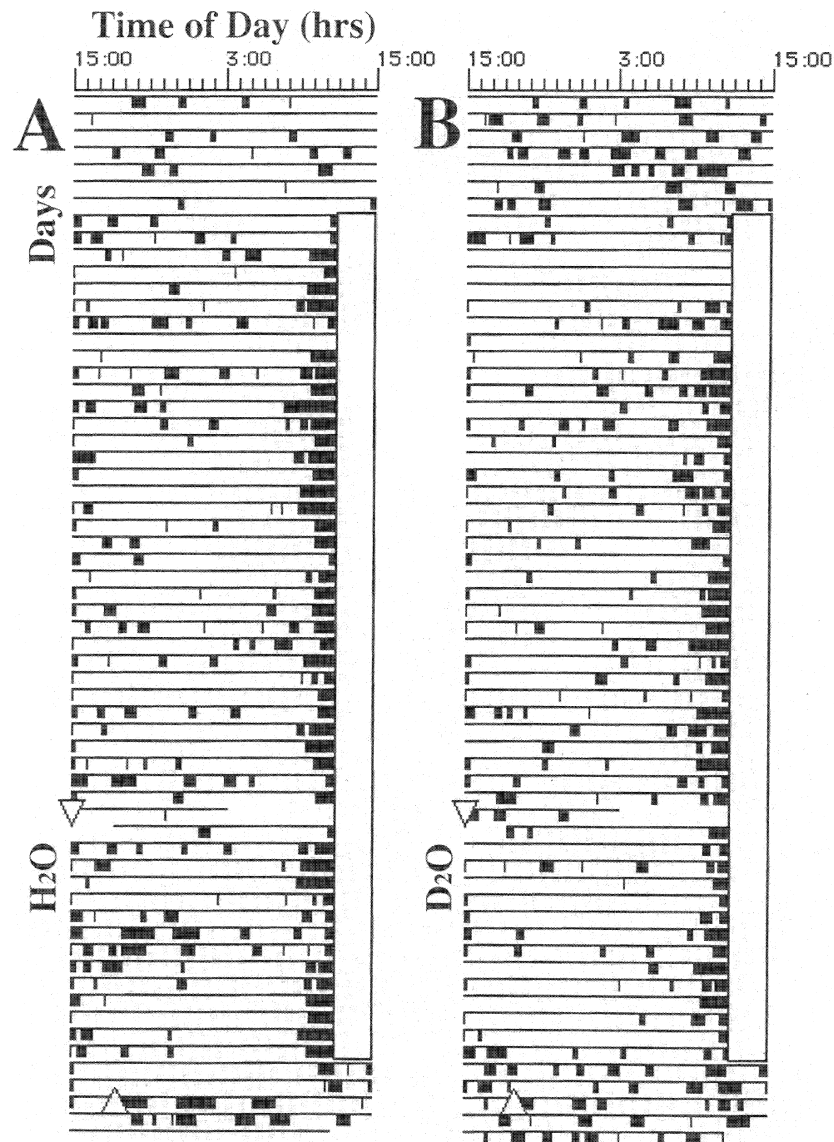


Fig. 5. Single-plotted actograms of food-bin activity from representative SCN-ablated rats provided with tap water (A) or D₂O (B). Mealtime during restricted feeding is indicated by the opaque vertical bar. No food was provided between the last meal of the schedule and the end of the D₂O treatment, denoted by the triangle. The onset of D₂O is denoted by the arrowhead. See Fig. 1 for other plotting conventions.

pacemaker, but that this is masked by the strength of the feeding zeitgeber. However, previous studies have clearly demonstrated in intact and SCN-ablated rats that the duration of FAA (i.e., the phase angle of entrainment) is significantly and predictably altered as the period of the feeding cycle is shortened or lengthened away from 24 h. (e.g. [2,19]). Restriction of food to a 2–3 h daily mealtime is therefore not a zeitgeber of sufficient strength to prevent changes in the phase angle of entrainment as the difference in period between pacemaker and feeding cycle is experimentally altered.

Slowing of periodic processes by D₂O is a remarkably ubiquitous and reliable phenomenon characteristic of plants and animals expressing biological rhythms with

intrinsic period lengths ranging from ultradian ($\ll 24$ h) to circadian (e.g. [10,13]). Thus, the demonstration of a clock-controlled rhythm that violates this general rule is of some interest. To our knowledge, the only other reported exception is the ultradian (2–3 h) rhythm of feeding behavior in SCN-ablated voles, which is also insensitive to D₂O at concentrations of 25% [11]. It is conceivable that oscillators driving vole ultradian feeding rhythms are homologous to food-entrainable oscillators in rats, although studies to establish this have not been done.

Insensitivity of food-entrained circadian rhythms to D₂O implies that at some level there is diversity in the molecular mechanisms underlying circadian timing in mammals. D₂O alters many fundamental aspects of cell function,

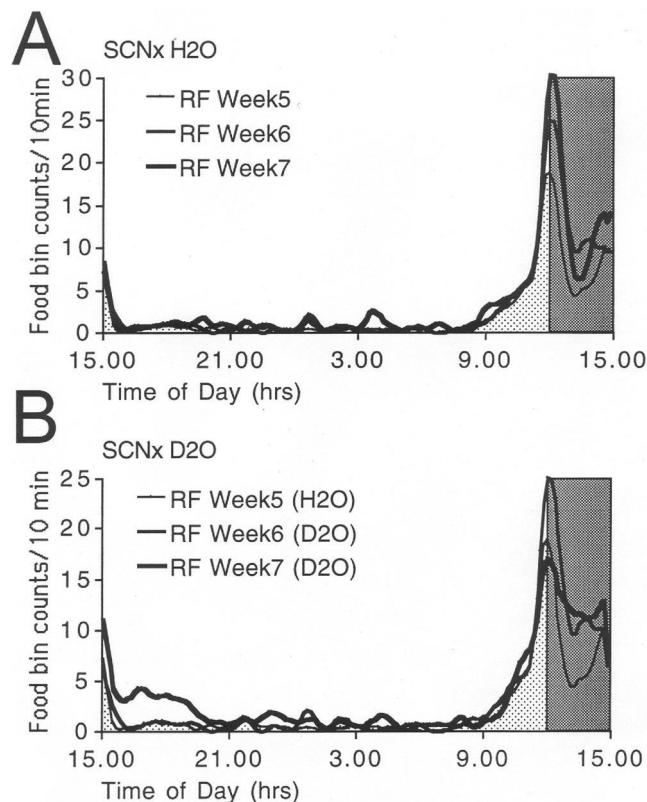


Fig. 6. Group mean average waveforms of food-bin activity from rats receiving tap water (A) or D₂O (B) during the last 2 weeks of restricted feeding. The thin line and stippling represent activity during the last week of water access. The heaviest line represents activity during the last week of D₂O treatment. The hatched vertical bar represents mealtime.

including reaction rates, diffusion rates, ion mobility, acidity and protein stability, in ways that presumably introduce a lag at one or more phases of the feedback loops regulating cyclic expression of clock genes in SCN clock cells. Failure of D₂O to alter food-entrained rhythms in rats and ultradian feeding rhythms in voles raises the possibility that the timing mechanism underlying these phenomena has a very different structure, e.g., it may be based on central or enteric neural networks with intercellular timing loops little affected by D₂O. Recent work suggests that FAA in mice is not affected by mutations of *clock*, a gene at the core of the light-entrainable SCN pacemaker [5]. D₂O may serve as an additional screen to identify putative food-entrainable circadian pacemakers from among those central and peripheral tissues that can be induced to oscillate with a circadian period independent of the SCN.

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References

- [1] M. Akashi, E. Nishida, Involvement of the MAP kinase cascade in resetting of the mammalian circadian clock, *Genes Dev.* 14 (2000) 645–649.
- [2] J. Aschoff, C. von Goetz, K. Honma, Restricted feeding in rats: Effects of varying feeding cycles, *Z. Tierpsych.* 63 (1983) 91–111.
- [3] A. Balsalobre, F. Damiola, U. Schibler, A serum shock induces circadian gene expression in mammalian tissue culture cells, *Cell* 93 (1998) 929–937.
- [4] A. Balsalobre, L. Marcacci, U. Schibler, Multiple signaling pathways elicit circadian gene expression in cultured Rat-1 fibroblasts, *Curr. Biol.* 10 (2000) 1291–1294.
- [5] E. Challet, J.S. Takahashi, F.W. Turek, Nonphotic phase-shifting in clock mutant mice, *Brain Res.* 859 (2000) 398–403.
- [6] G.J. Coleman, S. Harper, J.D. Clarke, S. Armstrong, Evidence for a separate meal-associated oscillator in the rat, *Physiol. Behav.* 29 (1982) 107–115.
- [7] S. Daan, C.S. Pittendrigh, A functional analysis of circadian pacemakers in nocturnal rodents: III. Heavy water and constant light: Homeostasis of frequency?, *J. Comp. Physiol.* 106 (1976) 267–290.
- [8] F. Damiola, N. Le Minh, N. Preitner, B. Kornmann, F. Fleury-Olela, U. Schibler, Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus, *Genes Dev.* 14 (2000) 2950–2961.
- [9] H.B. Dowse, J.D. Palmer, The chronomutagenic effect of deuterium oxide on the period and entrainment of a biological rhythm, *Biol. Bull.* 143 (1972) 513–524.
- [10] J.T. Enright, Heavy water slows biological timing processes, *Z. Vergl. Physiol.* 72 (1971) 1–16.
- [11] M.P. Gerkema, S. Daan, M. Wiblbrink, M.W. Hop, F. van der Leest, Phase control of ultradian feeding rhythms in the common vole (*Microtus arvalis*): The roles of light and the circadian system, *J. Biol. Rhythms* 8 (1993) 151–171.
- [12] M.E. Harrington, R.E. Mistlberger, Anatomy and physiology of the mammalian circadian system, *Principles and Practise of Sleep Medicine* 3 (2000) 334–345.
- [13] D.E. Logothetis, Z. Boulous, M. Terman, Lick rate and the circadian rhythm of water intake in the rat: Effect of deuterium oxide. *Annal N.Y. Acad. Sci.* 423.
- [14] R.E. Mistlberger, Circadian food anticipatory activity: Formal models and physiological mechanisms, *Neurosci. Biobeh. Rev.* 189 (1994) 171–195.
- [15] R.E. Mistlberger, B. Rusak, Circadian rhythms in mammals: Formal properties and environmental influences, *Principles and Practise of Sleep Medicine* 3 (2000) 321–333.
- [16] M. Oklejewicz, R.A. Hut, S. Daan, Effect of deuterium on the circadian period and metabolism in wild-type and tau mutant Syrian hamsters, *Physiol. Behav.* 71 (2000) 69–74.
- [17] S.M. Reppert, D.R. Weaver, Comparing clockworks: mouse versus fly, *J. Biol. Rhythms* 15 (2000) 357–364.
- [18] A.M. Rosenwasser, R.J. Pelchat, N.T. Adler, Memory for feeding time: Possible dependence on coupled circadian oscillators, *Physiol. Behav.* 32 (1984) 25–30.
- [19] F.K. Stephan, Limits of entrainment to periodic feeding in rats with suprachiasmatic lesions, *J. Comp. Physiol.* 143 (1981) 401–410.
- [20] F.K. Stephan, Phase shifts of circadian rhythms in activity entrained to food access, *Physiol. Behav.* 32 (1984) 663–671.
- [21] F.K. Stephan, Resetting of a circadian clock by food pulses, *Physiol. Behav.* 52 (1992) 997–1008.
- [22] K.A. Stokkan, S. Yamazaki, H. Tei, Y. Sakaki, M. Menaker, Entrainment of the circadian clock in the liver by feeding, *Science* 291 (2001) 490–493.
- [23] R.B. Suter, K.S. Rawson, Circadian activity rhythm of the deer-mouse, *Peromyscus*: Effect of deuterium oxide, *Science* 160 (1968) 1011–1014.

- [24] K. Yagita, H. Okamura, Forskolin induces circadian gene expression of *rPer1*, *rPer2* and *dbp* in mammalian rat-1 fibroblasts, FEBS Lett. 465 (2000) 79–82.
- [25] K. Yagita, F. Tamanini, G.T.J. van der Horst, H. Okamura, Molecular mechanisms of the biological clock in cultured fibroblasts, Science 292 (2001) 278–281.
- [26] S. Yamazaki, R. Numano, M. Abe, A. Hida, R. Takahashi, M. Ueda, G.D. Block, Y. Sakaki, M. Menaker, H. Tei, Resetting central and peripheral circadian oscillators in transgenic rats, Science 288 (2000) 682–685.