COPING with time is crucial to survival and indispensable for the adaptation of living organisms to predictable environmental events. For instance, because prey availability can be time-locked to limited periods of the 24-h light-dark cycle, mechanisms are needed to anticipate the precise moment at which they might be hunted (i.e., to learn associations between events important for survival and time of day) [see, for instance, (18)]. Furthermore, other mechanisms in the min or h range are necessary to determine the “giving-up time” (i.e., the moment of departure from a hunting ground or a patch) on the basis of the density of prey caught per unit of time (3,4,11). The former are endogenous oscillators with a circadian period (about 24 h), a high inertia, and independence from external events. Several data collected with lesion and transplant experiments suggested that the suprachiasmatic nucleus (SCN), located in the anterior hypothalamus (or the pineal gland in birds), is the neurological substrate of the circadian activity rhythm (54,62) [see also (39)]. They also proved that the signals sent by the SCN are humoral, rather than neural (20,38,54). Genetic determinants of the circadian rhythm begin to be unraveled as well: the Tau gene, whose mutation shortens or lengthens the endogenous rhythm of Mesocricetus auratus (38) and, in the fruit fly (Drosophila melanogaster), the Per and the Tim genes, which, when mutated, respectively modulate the circadian period of eclosion and activity, and the resetting of the circadian clock (20,27,28,34) [see also (12,19) for reviews].

Models developed since the late 1970s have described both the formal (15) and psychological structure of the interval timer that regulates the amount of time allocated to a particular activity (6). A temporal information-processing mechanism was proposed, that can be started, stopped, and reset at will, and is organized in 3 interacting levels. At the first of these (the “clock” level), a biological pacemaker is supposed to produce pulses that are gated to an accumulator by a switch. This accumulator is simply an “up counter” that stores pulses, whose number, \( n \), is a function of the Rate of the pacemaker (\( \lambda \)) times the Duration to be estimated. The second level includes a working memory, functionally equivalent to the accumulator, where \( n \) is transferred in the case of a delay between the end of a duration stimulus and the opportunity to respond (in time-discrimination procedures), and a reference memory where important durations, such as the durations reinforced in the past experience of the subject, are stored. Durations in reference memory are supposedly modulated by a multiplicative constant, \( K^* \), that might be equal to, smaller than, or greater than 1. Finally, a decision level compares online a duration sampled from reference memory at the beginning of a trial, \( n^* \), and the values taken by the accumulator, \( n \). If this last value is close enough to \( n^* \) (if it satisfies a similarity rule \( b \)), the decision is made to produce a temporal estimate. This model incorporates several sources of variance (at the level of the pacemaker, the switch, reference memory, or the response thresholds) allowing simulation of the behavior of living organisms, which are not as accurate as a mechanical clock (9,17).

The modular architecture of this model is compatible with localized disruptions at the level of one or the other component, as recent data from humans and rats have shown, and its functioning seems to depend upon striatocortical loops. More precisely, the substantia nigra, the striatum, and the hippocampus...
have been identified as the plausible neurological substrates of the pacemaker, the accumulator, and the working memory, respectively, whereas the frontal cortex is supposed to modulate the transfer of important durations to reference memory (32,33,35). Pharmacological studies also yielded valuable data concerning neurotransmitter systems involved in measuring time. For instance, the dopaminergic and cholinergic systems are thought to modulate clock speed (K) and memory functions (K*), respectively (30,31), whereas attentional processes indispensible to the transfer of impulses to the accumulator seem to interact with the noradrenergic system (35). More detailed accounts concerning the mechanisms of the interval clock or their neurological and pharmacological correlates can be found in Church, Meck and Gibbon (8), Hinton and Meck (21), or Meck (32).

Three main experimental strategies can be followed to study the relationship between the circadian rhythm and the interval timer. First, experiments were designed to record circadian rhythmicities of temporal discrimination or regulation of behavior. In such experiments, behavioral probes were introduced at different phases of the circadian cycle (13,43). Results usually showed that temporal regulation indices follow circadian rhythmicities (13). However, other effects were also described, rhythmicities being found only at the level of response rates, and not at the level of their temporal regulation (43).

Testing performance with temporal parameters close to the circadian periodicity is another strategy that might be fruitful in elucidating the relationships between timing mechanisms, because ‘‘anchor’’ effects might be expected at the fringe of the range of entrainment of the circadian oscillator. The FI schedule, where a reinforcer is delivered upon the first response emitted after a specified temporal interval has elapsed since the previous reinforcer delivery, is well suited to this experimental strategy. In this schedule, responses given during the interval are without consequence. Typically, the response pattern developed after a few training sessions can be described as a pause (after reinforcement), followed by progressively increasing response rates until the next reinforcer delivery (50). Usually, the duration of the postreinforcement pause (which expresses the measure of time within the interval) covers 50 to 70% of the duration of the fixed interval. The only data obtained with long FIs were reported by Dewes (10), who trained pigeons on FIs of 8 and about 28 h. Even if typical FI behavior patterns were described in both cases, such data did not shed light on the relationship between timing mechanisms, because of the unexplored gap between both FI values.

A third, and more promising, approach derived from the observation of activity bouts prior to periodic feeding in laboratory rats. With food available for 25 min every 24 h, rats developed an activity pattern that started about 2–3 h before food delivery (44). This anticipatory activity also appeared if food was made contingent upon an operant response (1,2,56), and was strikingly similar to the 28-h FI performance described by Dewes (10). Other reports also showed that, in a constant environment, anticipatory behavior strictly adjusted to the 24-h intermeal interval, whereas general activity continued to free-run, and that the respective phenomena were monitored by weakly coupled, but interactive, circadian clocks whose neural correlates could be identified (23,36,37,40,46,47,51–54). Furthermore, whereas SCN lesions in rats completely disrupted the circadian periodicity of general activity, food anticipation or performance on a 60-s FI were not impaired (22,54). However, even if FI behavior and food anticipation did not react to SCN lesions, this does not imply the commonality of neurological substrates, the more that anticipation behavior can be manipulated only within a range of entrainment, a property it shares with biological rhythms. Thus, even if food anticipation and behavior on FI look strikingly similar, they probably do not rely on identical mechanisms.

Further studies, where auditory cues predictive of mealtime were added in some conditions, suggested that the anticipatory pattern depended upon the interaction of three clocks. The first of them was a free-running circadian timer controlling the level of anticipatory activity, rather than the accuracy of mealtime estimation. In other words, the amount of anticipatory activity depended upon its phase relationship to free-running general activity. A second mechanism was a circadian food-entrainable timer that dissociated from the free-running clock under restricted feeding conditions. Finally, there was a cue-supported interval timer that could be arbitrarily reset and that was able to time multiple intervals concurrently, as upon the transition from a short to a long premeal cue (56).

Whereas food anticipation data were best explained appealing to circadian oscillators (48), time-place learning within a tighter span (60 min, with 4 response keys associated, each for 15 min, to a different location of food in the experimental environment) could best be accounted for by a stopwatch-like mechanism (i.e., by an interval clock with stop, restart, and reset properties) (61). These last experiments thus, argued for distinct mechanisms, depending upon the time span in force.

It follows from the preceding review that the relationship between biological and interval clocks usually appeared as the coexistence between two systems, on a ‘‘parallel’’ or an ‘‘either-or’’ mode. An alternative view suggested that an ‘‘integrated’’ collaboration between both clock systems might exist. Indeed, the connexionist version of the information processing model of the interval timer replaced the pacemaker by a set of biological oscillators coding durations by their phase, instead of numbers of ‘‘pulses’’ stored in reference memory (7).

Experimental strategies followed to uncover the relationship between both clock systems have been illustrated here, above. The most radical resorted to lesions within the central nervous system (22). A less invasive strategy will be followed here. Preliminary results from our work showed that rats exposed between 1 and 18 days of life to shifts of circadian Zeitgebers (the ‘‘1-18 rats’’) were, at adult age, deficient with respect to controls (C) on a Y-maze avoidance task (25,26). Because the precocious unsettling of the circadian system seemed to have long-lasting behavioral consequences, it was decided to compare 1-18 rats and untreated C rats on a temporal regulation task, the Peak Interval procedure (PI).

This schedule derives from the discrete-trial FI procedure, where intervals are not linked to each other, as in normal FI, but are separated by Inter-Trial-Intervals (ITIs). Furthermore, each interval is signalled by a stimulus (sound or light) that ends at reinforcer delivery. The transformation of a discrete-trial FI into a PI procedure requires two changes. A certain proportion of the trials (the peak trials) last much longer than the FI trials and stop automatically, without reinforcer delivery. Response rates averaged over these peak trials increase progressively with elapsed time, up to a maximum and decrease progressively thereafter. This response rate function is typically bell-shaped, with a small positive bias on the right slope. It might be considered as equivalent to a distribution of temporal estimates, from which two variables can be derived: peak rate (i.e., the highest response rate emitted during the trial) and peak time (i.e., the precise moment at which peak rate occurs). Peak time is considered as the estimate of T, the moment at which the reinforcer should have been delivered on FI trials. Performance previously obtained with this procedure also fit with the predictions of scalar timing theory (15). For example, peak time matches the duration of the fixed-
interval \( T \), and the coefficient of variation (CV, standard deviation/mean) of the response rate distribution remains constant whatever the duration of \( T \) (which, however, means that the absolute error increases with \( T \)).

Two hypotheses were advanced in the present work. The first one generalized the impairment observed in the Y-maze to other learning tasks in general. Thus, C rats were expected to be more accurate than their 1-18 counterparts. An alternative hypothesis posited that the precocious perturbation of the circadian system might have “aroused” all systems subserving the measurement of time, including the hypothetical interval timer, and that this “crossed sensitization” would favorably influence response timing. According to this hypothesis, 1-18 rats might be expected to be more accurate and sensitive to time than Cs. This last hypothesis, therefore, delineates a new approach of the interactions between systems involved in the measure of time.

**METHODS**

**Subjects and Chronobiological Treatment**

The subjects in this experiment were born in the rat colony of the Psychobiology Laboratory of the University of Louvain-la-Neuve, transferred to the animal quarters of the Experimental Psychology Laboratory of the University of Liège when they were 80 days old and tested later at the age of 100 days. The Psychobiology Laboratory of Louvain was equipped to manipulate circadian Zeitgebers. Liège provided the conditioning apparatus and expertise.

The genitors were Wistar rats bred under standard colony conditions (22°C, 12:12 L:D cycle, with lights on at 0800 h and 25 W illumination). Water and food were accessible ad lib. Multipara females were put with males in macronol cages for 10 days, after which the females were isolated in identical macronol cages. Only births occurring within a 24-h span were taken into account, and pups were randomly distributed between females (cross-fostering: each female was allocated 8 pups). The breeding cages were then equally distributed in 2 identical rooms. The first (A) was the control room where two cycles ran synchronously: a 12:12 light cycle (LC) (100 lx at 0800 h–0 lx at 2000 h), and a 12:12 temperature cycle (TC) (24°C at 0800 h–19°C at 2000 h). The second room (B) housed experimental subjects. The LC and TC were identical to those already described, except that their phase relation was 180° (12 h). Furthermore, they were simultaneously shifted 90° (6 h) every 54 h. As can be seen in Fig. 1, compared to Cs, experimental rats were exposed to “runaway” cycles, cumulated shifts delaying more and more the onset of a particular phase of the Zeitgebers (see arrows in Fig. 1). The experimental rats were exposed to this treatment from day 1 (birth) to day 18 (1-18 rats). Thereafter, all experimental rats were put under housing conditions identical to those of the C subjects.

At 80 days of age, rats were transferred to the University of Liège and housed in a colony room where illumination and temperature conditions close to those from the Louvain laboratory prevailed. The rats were put in individual macronol cages, on two metallic shelves facing each other. To equalize housing conditions, C subjects alternated on the shelves with 1-18 rats (C, 1-18, C, 1-18,..., etc.). Water and food were accessible ad lib and the door of the colony room was locked except for quasweekly food/water refill and cleaning, which took place each time at a different hour of the day and a different day of the week. When the rats were 96 days old, ad lib food was replaced by a daily amount of 5 g given at a different time each day. No food was provided on the eve of the shaping session.

**Apparatus**

The rats worked in 15 cubicles (40 × 40 × 45 cm), 5 per experimental room. The side walls of the cubicles were made of transparent perspex, the bottom and the top of aluminium grid with square perforations (1 × 1 cm). Ten of the cubicles had a front door. The other 5 could be accessed via a removable top. A pellet dispenser delivered 45 mg Noyes food pellets in a small food cup located on the left wall, 6 cm above the floor and 6 cm to the right of the front panel. A retractable lever was located 10 cm to the right of the food cup, 5 cm above the floor. No water bottle was available, because preliminary experiments showed that some rats were prone to develop polydipsia. Sounds (40 dB and between 3800 and 4200 Hz, depending on the cubicle) were delivered by a loudspeaker fixed on the top of each cubicle. These sounds were within the range of those well perceived by rats (24). Each cubicle was located in a bigger ventilated, heated (20–21°C), lighted (25 W bulb) and sound-isolated enclosure (70 × 60 × 160 cm). Each enclosure had a small observation window (22 × 22 cm) located on the front door. Monitoring of the experiment and recording of the data were controlled by personal computers.

**Behavioral Testing Procedure**

Behavioral experiments started when the rats were 100 days old. The subjects were maintained at approximately 85% of ad lib weight and received each day a supplementary food amount (12–14 g dry food chunks, depending on the subject) at 1630 h. Rats were group-caged at about 0900 h (5 rats per cage i.e., those working in the same laboratory room) and isolated for the night.

![FIG. 1. Schematic representation of the chronobiological treatment of Experimental (Exp, top) and Control rats (Ctr, bottom). Darkness is represented by black surfaces. For other details, see text.](image-url)
After being weighed to the nearest tenth of a gram, all rats were transferred to the laboratory rooms at the same time (about 1140 h). These rooms were dimly illuminated and kept at 20–21°C. Because 15 experimental cubicules were available, rats worked in 2 successive shifts, the first starting at about 1145 h and the second at 1400 h. The second session ended latest at 1615 h.

**Shaping and consolidation of the response.** The rats were semi-automatically shaped on a discrete-trial FI 1-s procedure (FI 1-s), with an average 2.5-s ITI (range 2–3 s). If necessary, the experimenter could dispense food pellets with a microswitch. As soon as the operant response was mastered (usually after receiving from 30 to 50 food pellets, depending on the subject), rats were transferred to an automatically monitored discrete-trial FI 1-s schedule for an additional 25 pellets. After completion of this schedule, each subject was returned to the animal quarters, put in the familiar individual cage and given a small amount of additional food (about 5 g). On the second day, each rat performed successfully 2 sessions limited to 25 reinforcers each: a discrete-trial FI 1-s session, as on the previous day, and a discrete-trial FI 6-s session with an average 6.5-s ITI (range 6–7 s). The third day, all subjects performed a discrete-trial FI 20-s session limited to 20 reinforcers, with a 20-s ITI.

**PD training.** Two types of conditions were run in different phases of the experiments. The first was a 20-s baseline condition where successive sessions were run at the 20-s criterion. The second was a triangular-cycles condition, where criterion duration changed from one session to the next. Two types of triangular cycles were explored: 3-valued 10–20–30–20–10-s cycles and 2-valued 10–30–10-s cycles. For example, in the first phase of Experiment 2, 6 complete 10–20–30–20–10-s cycles were successfully performed. As in cyclic PI, criterion values changed from one session to the next, influence of criterion n-1 on performance at criterion n was common. For clarity and convenience, these effects were labeled “proactive,” even if those recorded when the 20-s criterion was in force might have been labeled “retroactive,” because performance typical of the initial 20-s baseline was changed. PI data were analyzed over intervals equal to twice the FI value, T. Behavioral indices were, thus, computed on the first 20–40–60-s of the peak trials, when the FI criteria were 10, 20, or 30 s, respectively. Measures were obtained for each session and each rat. Whatever the value of T, peak trials were semairandomly intermixed within FI trials, with the restriction that no more than 3 peak trials could occur successively. Whatever the condition, each session included 63 FI and 27 peak trials (i.e., 30% of trials were peak trials). ITIs lasted in each case for 30 s, on average (range: 15–45 s). At the PI 20-s baseline condition, peak trials lasted for 70 s (range: 65–75 s). On triangular cycles, they lasted for 80 s (range: 75–85 s).

**Data Analysis**

Responses were collected in successive 1-s segments of FI and peak trials. Data from the first trial of each PI session were discarded, but results from all remaining trials were taken into account.

Two behavioral indices were obtained from PI data, peak time and the coefficient of variation (CV) of the response-rate function. Peak times were medians obtained by iteration, as described in Roberts ([45], page 244). First, a median peak time was obtained from 20-, 40-, or 60-s intervals. Thereafter, a new median was computed, taking the first median as the center. For example, if the first median obtained from the 60-s interval was located at 20 s, the second median took a 40-s interval into account. If the second median was located within a ± 0.5-s range around the first, computation stopped. If not, a third median was computed from the interval defined by the second median, and so on. The CV was the ratio between the standard deviation and the mean of the response-rate function. It measures in relative terms the spread of the response-rate function. In other words, it measures the sensitivity to time (more or less peaked response-rate function), whereas peak time reflects the accuracy of timing performance (matching with T). Furthermore, microdata [i.e., the start (s1), stop (s2), spread (d) and middle (m) of individual peak trials (8)] were obtained from the last 5 sessions of the PI 20-s baseline.

Three more traditional indices were computed from the FI trials of the session: the index of curvature, IC (14), the duration of the postreinforcement pause, and the response rate (RR). The IC was computed from the cumulative response frequencies obtained in successive segments of the FI. It yields high positive values in the case of a good temporal regulation of responses (when most responses are located at the end of the interval, in the last segments) and a zero value in the absence of response timing, when responses occur at a constant rate throughout the interval. Negative values can be obtained if responses at the beginning of the interval outweigh those at its end. The IC is, thus, a measure of the location of responses within the interval (i.e., of response timing). The postreinforcement pause is the time elapsed between reinforcement and the first response given during the subsequent FI, averaged over the FI trials of the session. The RR is the ratio between the total number of responses given on FI trials, divided by the total duration of the FI trials of the session. It is expressed as number of responses per min (R/min).

Data from baseline sessions were analyzed with an ANOVA for repeated measures, with sessions as the within-group factor. Correlations (Spearman r) between indices derived from individual peak trials (s1, s2, d, and m) were computed to evaluate the sources of variance at play, in light of the temporal information-processing model and scalar timing theory. Finally, group-related differences between s1, s2, d, and m were evaluated with the Student’s t-test.

In the case of a cyclically changing temporal criterion, the 5 dependent variables were subjected to an ANCOVA with an harmonic concomitant variable. This analysis enabled the comparison between groups of subjects, after fluctuations imposed by the procedure were neutralized. Finally, the standard error of the mean (SEM), which is equal to the ratio between the standard deviation and the square root of the number of subjects n, was computed for each variable and each session.

**EXPERIMENT 1**

**PEAK PROCEDURE 20-s TRAINING (SESSIONS 1 TO 40)**

A 20-s peak procedure training started on the fourth day. One of the groups (1-18 or C) started at about 1145 h, the other at 1400 h. The second session was completed latest at 1615 h. This scheduling ensured that performances were obtained before the increase in general activity, typical at the end of the afternoon. All rats were kept in the laboratory until 1625 h, and then returned to their individual cages in the animal quarters to receive their supplementary daily food amount. To counterbalance hypothetical session order effects, the group that performed first on the first day performed second the next day, and so on. All statistical analyses were restricted to the last 10 sessions of the 40-session series.

**RESULTS**

Daily average peak times (top) and CVs (bottom) are presented in Fig. 2. Peak times increased and the CV values de-
increased before reaching stability, from about 25 to 30 training sessions onward. Peak times closely tracked the 20-s duration criterion. Although 1-18 Rats had slightly lower peak times than Cs (19.4 vs. 19.7, respectively), no significant between-group difference could be found ($F(1,28) = 0.22, p = 0.641$). Peak times remained approximately constant, because neither a session nor a Group $\times$ Session interaction reached statistical significance [$F(9,252) = 0.78, p = 0.636$ and $F(9,252) = 0.95, p = 0.482$, respectively]. Similar conclusions could be reached for the coefficient of variation (CV), which was close to 0.42 for 1-18 and to 0.45 for C rats [$F(1,28) = 1.96, p = 0.173$; $F(9,252) = 1.28, p = 0.247$; $F(9,252) = 0.85, p = 0.574$, respectively, for Group, Session, and Group $\times$ Session interaction effects]. However, 1-18 rats undermatched a CV value of 0.45 from session 27 on, whereas Cs had not reached this criterion at the end of the session series. For both dependent variables, dispersion of the data were rather small, as can be inferred from the SEM measure, without clearcut between-group differences.

Figure 3 presents average postreinforcement pauses (top), ICs (middle) and RRs (bottom). Pause, IC, and RR values increased over successive sessions as timing behavior improved and seemed to reach stability from about 30 sessions on. Furthermore, for pauses and ICs, divergence between groups appeared after 10 to 15 sessions and tended to increase with further training. Experimental rats systematically overmatched a 5-s pause duration from session 25 on, whereas Cs remained around this value. Similarly, an IC of 0.20 was overshot after 16 sessions in 1-18 rats, vs. 25 sessions in Cs. Between-group differences in RRs appeared more precociously and remained constant. Experimental rats seemed to be more sensitive to time than Cs subjects because their postreinforcement pauses were, on average, longer (6.24 s) than those of Cs (4.88 s). However, this between-group difference was not significant [$F(1,28) = 2.74, p = 0.108$]. Furthermore, neither Session nor Group $\times$ Session effects reached significance [$F(9,252) = 1.38, p = 0.195$; $F(9,252) = 1.16, p = 0.322$, respectively]. ICs followed the same trend. They were on average higher for 1-18 rats (0.308) than for Cs (0.244), but this difference was not significant [$F(1,28) = 2.79, p = 0.106$]. As was the case for the pause, neither Session nor Group $\times$ Session effects were significant [$F(9,252) = 0.78, p = 0.636$ and $F(9,252) = 0.95, p = 0.482$, respectively]. Although experimental rats had, on average, higher RRs than Cs, as can be seen at the bottom of Fig. 3, this difference was not significant [$F(1,28) = 0.709, p = 0.411$]. SEMs of pause durations and ICs tended to be smaller for 1-18 rats, whereas such a difference was not found for RRs.

**FIG. 2.** Peak times (top) and coefficients of variation (bottom) of 1-18 (■) and Control rats (□) on a 20-s peak interval (PI) procedure. Data are daily group means with their standard errors (SEMs).

**DISCUSSION**

Data obtained in Experiment 1 did not confirm that preweaning shifts of circadian Zeitgebers might disrupt the hypothetical interval timer and impair response timing. On the contrary, 1-18 rats tended to be more sensitive to time, although between-group differences did not reach statistical significance. The lack of significant differences could be due to the relationship between the dam and their pups, as if dams acted as “buffers” between the young and the atypical environment. In other words, it might be suspected that the dams were not reliable mediators of the Zeitgeber manipulations, due to the high inertia of their own circadian system. This speculation was, however, not confirmed. First, data from a complementary experiment, where waterspout licking was recorded, confirmed that drinking was entrained by the phase shifts of the light Zeitgeber, as can be seen in Fig. 4 displaying data from 3 experimental dams from days 2 to 17 after birth of the pups. Because it is well known that, under LD conditions, ambulation is synchronized with drinking [as shown, for instance, in Figs. 2 or 8 from Usui et al. (59)], and as parts of pup care (such as licking, grooming, stroking, displacement from and retrieval to the nest) typically take place when dams are active, activity changes could not remain unnoticed by the young. Second, even if mother care was influenced by the atypical Zeitgebers, there is no reason to suspect that 1-18 rats were not appropriately cared for. Indeed, other data suggested that the average amount of care behavior did not significantly differ between experimental and Cs dams, even if “qualitative” changes in the phase and amplitude of these behaviors were found (49). In summary, because it has long been well known that dams are Zeitgebers to their immature offspring, even at fetal stages (41,55), it seems highly plausible that while mediating the atypical Zeitgeber influence, they somehow changed the “receptivity” of their pups to time. Finally, the best performance indices were found in 1-18 rats, whose response rates were, on average, higher than those of Cs rats.
The trends in our data, in fact, were better fitted to the second hypothesis, according to which precocious manipulation of the circadian system might have induced a sensitization of the young subjects to temporal parameters in general. The behavioral consequences of this change in sensitivity would progressively show up later on, as a consequence of the repeated interaction between the subject and a particular temporal contingency. Indeed, Figs. 2 and 3 clearly show that group-related differences were absent early in training and progressively developed thereafter. It was further speculated that the simple baseline peak procedure might not have been a test sensitive enough to "uncover" the gain due to this preweaning sensitization. A more demanding PI was, thus,
chosen, where criterion durations changed cyclically from one session to the next. Two “triangular” cycles were successively run (10-, 20-, 30-, 20-, 10-s PI procedures) before 20-s and 10-, 20-, 30-, 20-, 10-s redeterminations took place.

It was hypothesized, first, that differences between 1-18 and C subjects would appear and reach statistical significance, at least during the first cycle involving 3 duration criteria. Second, as the discrimination between 10 and 30 s on the second cycle is less difficult than the previous one (between 10, 20, and 30 s), group-related differences were expected to fade out. Third, it was expected that the 20-s redetermination would replicate data from Experiment 1, without significant differences between groups, despite an average superiority of the 1-18 rats. Finally, differences were expected to appear again during the 10-, 20-, 30-, 20-, 10-s redetermination, maybe to a lesser extent.

EXPERIMENT 2

PROCEDURE

The same groups of rats were run in their familiar conditioning cubicles and exposed to 4 successive subexperiments. The first 2 conditions (A and B) run triangular-cycle Pls. The last two (C and D) were redeterminations (20-s baseline and cyclic 10-, 20-, 30-, 20-, 10-s PI).

(A) Cyclic Peak Procedure 10-, 20-, 30-, 20-, 10-s (Sessions 41–65)

Rats were exposed to a daily shift in the criterion duration. After a session on a 10-s schedule, they performed on 20-s (next day) and then on a 30-s peak procedure on the third day, before reverting back to 20 and 10 s, and so on. Six cycles were completed. All other conditions were identical to those from Experiment 1.

(B) Cyclic Peak Procedure 10-, 30-, 10-s (Sessions 66–77)

Experimental conditions were as just described, except that the intermediate criterion (20 s) was dropped. Six complete cycles were run.

(C) Peak Procedure 20-s Redetermination (Sessions 78–89)

Experimental conditions were identical to those described for Experiment 1, except that only 12 sessions were run.

(D) Triangular Cycle Peak Procedure 10-, 20-, 30-, 20-, 10-s Redetermination (Sessions 90–102)

Experimental conditions were identical to those from condition (A), except that only 3 cycles were run.

RESULTS

(A) 10-, 20-, 30-, 20-, 10-s Cyclic Peak Procedure

The top left (A) of Fig. 5 presents peak time and CV values. As can be seen, mean peak times fluctuated according to the criterion. However, strong proactive interference effects were present, because 10- and 30-s performances overmatched and undermatched, respectively, their criterion duration. Furthermore, 20-s peak times also fluctuated, seemingly influenced by the preceding criterion. The CV also fluctuated with T, but remained within a small range. Overall, absolute between-group differences were similar to those found in Experiment 1, but they became statistically significant. Although average differences were very small, peak times from 1-18 rats matched more closely criterion value, \( F(1,721) = 7.09, p < 0.01 \), and their CVs were on average lower than those from C subjects [0.418 vs. 0.452, respectively; \( F(1,721) = 34.07, p < 0.0001 \)]. Comparatively to the PI 20-s baseline, SEMs of 1-18 rats grew even smaller, whereas those of Cs tended to remain as they were previously. The bottom left of Fig. 5 shows mean postreinforcement pause and IC data. As depicted in the Fig., 1-18 rats paused longer \( [F(1,721) = 77.97, p < 0.0001] \) and obtained higher ICs than Cs \( [F(1,721) = 90.60, p < 0.0001] \). Proactive interference effects were found at the level of pause duration, as was the case for peak times. Pauses at 20-s were shorter after 10 s than after the 30-s schedule. IC values also were influenced by the cycle, but remained more stable from one T value to the next. Overall, pause durations, as well as IC values, tended to increase with further training. Trends in response rates were similar to those of the preceding experiment and are not shown. Within-group variability (SEM)s decreased mostly in the 1-18 rats, as comparison between Fig. 3 and the bottom of Fig. 5 shows.

(B) 10-, 30-, 10-s Cyclic Peak Procedure

Peak times and the CVs can be seen at the top right (B) of Fig. 5. As previously, 1-18 rats seemed to be more accurate and
sensitive than C rats. However, between-group differences in the value of peak time were just short of significance, $F(1,373) = 3.81, p < 0.052$, contrary to differences in the values of the CV (1-18: 0.409; C: 0.343), $F(1,373) = 25.02, p < 0.0001$. The other dependent variables are displayed at the bottom right of Fig. 5. Here, again, pauses were longer and ICs were higher for 1-18 subjects and, contrary to expectations, between-group differences remained significant, $F(1,373) = 31.5, p < 0.0001$, and $F(1,373) = 47.42, p < 0.0001$, respectively. Within-group variability (SEMs) remained globally similar to values found in the preceding phase of the experiment.

(C) Peak 20-s Redetermination

Peak times and CV values can be found at the top left (C) of Fig. 6. As expected, between-group differences in peak time values were not significant, $F(1,27) = 0.01, p = 0.908$. However, peak times increased slightly over the successive sessions series, $F(11,297) = 4.96, p < 0.0001$. CVs from 1-18 rats were again smaller than those of Cs, but this difference was just short of significance, $F(1,27) = 3.91, p < 0.058$. Nevertheless, CV progressively decreased with continued training, $F(11,297) = 28.33, p < 0.0001$. SEMs remained as previously.

The bottom left of Fig. 6 shows that between-group differences between postreinforcement pauses (7.25 vs. 6.20 s, respectively) and ICs (0.360 vs. 0.289, respectively) remained clearcut. However, as was the case during the first 20-s baseline, between-group differences were not significant, $F(1,27) = 2.15, p = 0.153$, and $F(1,27) = 2.66, p = 0.115$, respectively. Increases of pause duration and IC values over sessions were significant, $F(11,297) = 4.80, p < 0.0001$, and $F(1,27) = 6.60, p < 0.0001$, respectively. Between-group differences in response rates were similar to those previously described and nonsignificant. SEMs remained smaller for 1-18 rats.

(D) Peak 10-, 20-, 30-, 20-, 10-s Redetermination

The top right part (D) of Fig. 6 displays peak times and CV values. As expected, between-group differences in peak time were not significant, $F(1,360) = 0.02, p = 0.878$. However, CVs from 1-18 rats were significantly shorter than those of C rats, $F(1,360) = 38.39, p < 0.0001$. As was the case over the first 10-, 20-, 30-, 20-, 10-s cycle, pauses remained longer and ICs higher for the experimental group [$F(1,360) = 43.70, p < 0.0001$, and $F(1,360) = 50.23, p < 0.0001$, respectively]. These differences are depicted at the bottom right of Fig. 6. Proactive interference effects similar to those recorded during the first 10-, 20-, 30-, 20-, 10-s cycles showed up again, both at the level of average peak time and postreinforcement pause. Response rate differences were as those preceding and did not reach significance. SEMs remained within ranges found during phase (C) of the experiment.

GENERAL DISCUSSION

The present experiments aimed to test if the precocious disruption of a typical circadian Zeitgeber sequence could influence a response-timing performance learned in adulthood. The answer was positive, because significant between-group differences were found. Contrary to expectations based on previous results (25,26), the precocious change in phase and period of light and temperature Zeitgebers had a favorable influence on the estimation of short durations at adulthood. However, a particular test was needed to uncover these effects. Although average differences in favor of 1-18 rats appeared from about 10 to 15 training sessions on the first PI 20-s baseline, they reached significance only after the task requirements had been upgraded, where, instead of performing on a steady-state routine, all rats had to cope with daily changes in the temporal criterion of the task. Steady-state behavior seemed, thus, not to be a test sensitive enough to reveal the effects of the precocious manipulations of the circadian clock. It might, however, be contended that the cycling procedure was not the necessary condition for between-group differences to appear. Indeed, because between-group differences progressively increased over the PI 20-s training sessions, going on with the baseline training might have been eventually sufficient to highlight significant between-group effects. Deciding between these alternatives requires further experimentation.

The small between-group difference in the central tendency of temporal estimates (peak times) were not overwhelming at the
first 10-, 20-, 30-, 20-, 10-s cycle. Furthermore, this difference was just short of significance at the 10-, 30-, 10-s cycle and not significant at all at the 10-, 20-, 30-, 20-, 10-s redetermination. However, in agreement with the sensitization hypothesis, 1-18 rats were more receptive to time than Cs. Furthermore, between-group differences in the CV as well as the postreinforcement pause and IC on FI trials remained significant over the complete cycle sequences. This dissociation between peak time and the 3 other indices is most interesting because these indices concern different aspects of adaptation to time. In this respect, a comparison between FI and PI schedules might be enlightening. It has been suggested that the FI performance is equivalent to the left half of the response-rate function of the PI procedure (20). The postreinforcement pause and the IC thus can be taken as sensitivity indices, as is the CV. However, these indices are not redundant. Whereas the CV demarcates the central 66% of the distribution of temporal estimates, the postreinforcement pause indexes the very first response that follows the distribution of the reinforcer [see (26,35) for a discussion of this and related indices]. The IC takes into account the fine grain of behavior (here with a 1-s resolution), as time goes by in the fixed interval. This latter index is probably the most refined measure of sensitivity to time. Because the FI duration approximately matches the reinforced duration, no central tendency index can be derived from FI performance. Peak time was, thus, the only accuracy measure that could be obtained from the present set of data. Furthermore, peak time and the CV are not related to each other as are the postreinforcement pause and the IC (both sensitivity indices). Indeed, a same peak-time value is compatible with different CVs (more or less peaked response-rate functions around the same peak time).

Because clearcut and persistent differences were found at the level of the CV, the pause duration, and the IC, the question was first to check if these data did not derive from some methodological bias and, if such bias might be discarded, to explain why 1-18 rats were more sensitive to time than Cs. In particular, it might be asked if changes at the level of one or the other component of the temporal information-processing model (6) might account for the observed effects. Both issues will be successively discussed.

Because SEMs were larger for Cs than for 1-18 rats over Experiment 2, the question of a sampling hazard might be raised. Indeed, subjects from the C Group might, by chance, have been less homogeneous than their experimental counterparts. In other terms, some C rats performed as 1-18 subjects, whereas some others were clearly impaired with regard to the experimental group. This lesser within-group homogeneity can, however, not be responsible for patterns of effects. Indeed, a partial overlap between performances from both groups, rather, should have led to the absence of significant differences between 1-18 and C rats. Second, within-group variability was higher for those subjects that were not exposed to the unsettling Zeitgeber shifts, whereas it became smaller in 1-18 rats, a paradox that seems counterintuitive. Third, as could be seen in Figs. 2 and 3, between-group differences in mean values and SEMs were not present from scratch. They, rather, developed slowly over successive PI 20-s training sessions. Had sampling hazards been determinant, between-group differences might have been expected to appear much more precociously. This late splitting between samples indicated that the interaction between reinforcement contingencies and the subjects was necessary for the recorded effects to appear, a view that is compatible with the sensitization hypothesis. In other respects, the intuitive claim of an inverse relationship between response rates and performance indices in FI was disproved. Results confirmed the relative independence between both measures [see in (42)] in showing that higher response rates were associated to better response timing in 1-18 rats. In other words, high response rates do not necessarily induce small pauses and low ICs and, by analogy, large CVs in PI. 

Trying to explain between-group differences in light of the temporal information-processing model, the different components of the mechanism will be successively reviewed. First, changing the value of the multiplicative memory constant K* should induce shifts at the level of peak time. Indeed, a smaller \( K^* \) is supposed to shift peak time to the left, whereas a higher \( K^* \) should produce the opposite effect. However, because all durations transferred to reference memory undergo the same multiplication, the ratio between the coefficient of variation of the response-rate distribution and peak time remains constant. Changes in \( K^* \) do not influence the value of the CV. In other terms, variance linked to the transfer of temporal information to reference memory is scalar and changes in \( K^* \) are not supposed to influence the spread of the distribution of temporal estimates. Because changes induced by the chronobiological treatment produced the opposite effect (no change in peak time, and a decrease of the CV), memory variance cannot be taken into account to explain timing behavior of 1-18 rats. In other respects, the proportionate increase in 20-s peak time values over successive conditions, is probably a sequel of cycling and remains to be elucidated.

Two other hypotheses are available. The first concerns the pacemaker rate, \( \lambda \). A fast pacemaker (smaller interpulse intervals) might lead to a more accurate measure of time than a slow one, because the resolution of the metric is finer in the former case (for instance, 1 h is more accurately measured in s than in min). Because it was suggested that pacemaker rate is sensitive to arousal induced, for instance, by the intensity of the stimulus whose duration should be discriminated (60), it follows that pacemaker rate of the 1-18 rats might have been reactive to "arousal-like" properties of the increased sensitization to time. However, absolute pacemaker rate cannot be quantified, because no direct measure is available [see, however (57,58)]. Furthermore, according to scalar timing theory, changes in pacemaker rate can be inferred only from experimental settings where subjects are their own controls, and not from between-group comparisons. For instance, it has been shown that metamphetamine increases clock speed because peak times of rats were shifted to the left, relative to a condition without drug (30). The scalar timing model also assumes that variance linked to the pacemaker rate is scalar, as was the case for memory variance. Therefore, fluctuations in pacemaker rate should not influence the CV of the response-rate distributions, which can be superimposed when relative response rates are plotted against relative elapsed time. Because the experimental design followed here did not allow measure of pacemaker rate, and because similar peak times were associated to between-group differences in the CV values, this variable cannot be retained as a tentative explanation of the differences between 1-18 and C rats. In other respects, assuming that the precocious manipulation of the circadian Zeitgeber might have changed the pacemaker rate does not imply that the pacemaker is an oscillator, as suggested by the connectionist version of the temporal information-processing model (7). Such a suggestion would further require that the circadian Zeitgeber manipulation did change the period of the biological oscillators, a step that cannot be followed here, on the basis of available data.

The last hypothesis concerns the decision level of the model and the response threshold(s) controlling response output. The response rule supposed to control responding is not absolute, but relative. A decision is made to produce a temporal estimate when the subjective discrepancy between \( n^* \) and \( n \) is smaller than a
proportion of the reference memory value for reinforcement \((n^*/n - n < b)\). Changes in the threshold \(b\) thus directly modulate the spread of the distribution of temporal estimates. A small \(b\) will lead to a more peaked distribution of temporal estimates than a large one. Indeed, a small \(b\) value can be obtained only if there is a small difference between \(n^*\) and \(n\). By analogy, and because FI performance can be considered as equivalent to the left half of the response rate distribution \((29)\), it might be suggested that a small \(b\) also induced longer postreinforcement pauses and higher ICs. In the present case, it is as if 1-18 rats did use smaller response thresholds (smaller \(b_s\)) than C subjects. It also is as if 1-18 rats could reach a preset sensitivity level after a shorter training time than C, as can be seen for pause duration and IC variables in Fig. 3.

Finally, it might be asked if the limited sample of microdata collected over the last 5 sessions of the PI 20-s baseline corroborates this hypothesis. It is well known that the bell-shaped response rate function in PI derives from the summation of individual peak trials whose response pattern can be described as a step function: as a trial starts, animals first do not respond or give only a few responses, followed by a rather abrupt transition to a high response state (the run), which ends again, rather abruptly, to give way to a second low-response state (break-run-break pattern). Four behavioral indices can be derived from individual peak trials: the start \(s_1\), stop \(s_2\), spread \(d\), and middle \(m\) of the response run. Further, correlations between these indices allow detection and sources of variance at work. For instance, a fixed threshold \(b\) and a variable memory \((n^*)\) lead to a positive correlation between \(s_1\) and \(s_2\), whereas a fixed memory and a variable threshold produce a negative correlation between them. Correlation patterns previously obtained from groups of pigeons or rats exposed to the PI procedure reflected variance at the level of the memory for duration \((n^*)\), as well as at the level of the threshold \(b\) \((5,8,16)\). The question was, thus, to check if correlation patterns obtained here were close to or different from those obtained previously and, in particular, if between-group differences might be found.

Correlation patterns were computed on data selected according to criteria defined in Church et al. \((8)\) (i.e., after discarding trials in which \(s_1\) was higher or equal to, and \(s_2\) smaller than criterion duration \(T\)). They were coherent with those published previously. Indeed, positive correlations were found between \(s_1\) and \(s_2\), \(s_1\) and \(m\), \(s_2\) and \(d\), \(s_2\) and \(m\) and \(d\) and \(m\), whereas \(s_1\) and \(d\) were negatively correlated, which tends to indicate that sources of variance typical of performances congruent with the scalar model were at work. Furthermore, between-group differences in correlation values were not found. However, between-group comparison of start, stop, spread, and middle values showed that, at the end of the PI 20-s baseline, 1-18 rats started \((8.50 vs. 7.27 s)\), had a smaller spread \((22.23 vs. 23.76 s)\) and a more accurate middle than Cs \((19.62 vs. 19.15)\). These differences were significant (Student \(t\)-test: \(p < 0.001\) in each case). Stops did not significantly differ, although, on average, 1-18 rats ended their response runs earlier than Cs.

Taken together, these data plead again in favor of Zeitgeber manipulation modulating sensitivity to time (i.e., decreasing response threshold \(b\)). Thus, precocious manipulation of the circadian clock seemingly induced an improved sensitivity to time, which crossed the divide between biological rhythms and training-dependent adaptations to “artificial” short durations. Such an effect is compatible with any interaction mode — “either-or,” “parallel,” and “integrated” — between components of both clock systems. Data obtained here therefore highlighted a limited “anchor” point between circadian and interval clocks that did not unsettle the interval timer mechanisms. However, several questions remain pending. First, what about the temporal gap between chronobiological treatment and testing? Would more precocious testing have yielded similar results? Second, is the “sensitization” described here truly limited to time? Indeed, Zeitgeber shifts might have induced a sensitization to change, itself. Finally, do the data not merely reflect the effects of some peculiar precocious contextual “enrichment”? The present and previous data cannot answer these questions and substantiate these more general hypotheses. Further experiments are, thus, needed to validate or challenge this new approach to the relationship between systems subserving the measure of time.

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