

## INTERNAL DESYNCHRONIZATION OF CIRCADIAN RHYTHMS AND TOLERANCE TO SHIFT WORK

Alain Reinberg<sup>1</sup> and Israel Ashkenazi<sup>2</sup>

<sup>1</sup>*Unité de Chronobiologie, Fondation A. de Rothschild, Paris, France*

<sup>2</sup>*Sackler Faculty of Medicine, Department of Human Molecular Genetics and Biochemistry, Tel Aviv University, Tel Aviv, Israel*

Intolerance to shift work may result from individual susceptibility to an internal desynchronization. Some shift workers (SW) who show desynchronization of their circadian rhythms (e.g., sleep-wake, body temperature, and grip strength of both hands) exhibit symptoms of SW intolerance, such as sleep alteration, persistent fatigue, sleep medication dependence, and mood disturbances, including depression. Existing time series data previously collected from 48 male Caucasian French SW were reanalyzed specifically to test the hypothesis that internal synchronization of circadian rhythms is associated with SW intolerance and symptoms. The entry of the subjects into the study was randomized. Three groups were formed thereafter: SW with good tolerance ( $n = 14$ ); SW with poor tolerance, as evident by medical complaints for at least one year ( $n = 19$ ); and former SW ( $n = 15$ ) with very poor tolerance and who had been discharged from night work for at 1.5 yr span but who were symptom-free at the time of the study. Individual and longitudinal time series of selected variables (self-recorded sleep-wake data using a sleep log, self-measured grip strength of both hands using a Colin Gentile dynamometer, and oral temperature using a clinical thermometer) were gathered for at least 15 days, including during one or two night shifts. Measurements were performed 4–5 times/24 h. Power spectra that quantify the prominent period ( $\tau$ ) and  $t$ -test, chi square, and correlation coefficient were used as statistical tools. The mean ( $\pm$ SEM) age of SW with good tolerance was greater than that of SW with poor tolerance ( $44.9 \pm 2.1$  yrs vs.  $40.1 \pm 2.6$  yrs,  $p < .001$ ) and of former SW discharged from night work (very poor tolerance;  $33.4 \pm 1.7$ ,  $p < .001$ ). The shift-work duration (yrs) was longer in SW with good than poor tolerance ( $19.9 \pm 2.2$  yrs vs.  $15.7 \pm 2.2$ ;  $p < 0.002$ ) and former SW ( $10.7 \pm 1.2$ ;  $p < .0001$ ). The correlation between subject age and shift-work duration was stronger in tolerant SW ( $r = 0.97$ ,  $p < .0001$ ) than in non-tolerant SW ( $r = 0.80$ ,  $p < 0.001$ ) and greater than that of former SW ( $r = 0.72$ ,  $p < .01$ ). The mean sleep-wake rhythm  $\tau$  was 24 h for all 48 subjects. The number of desynchronized circadian rhythms ( $\tau$  differing from 24 h) was greater in non-tolerant than in tolerant SW (chi square = 38.9,  $p < .0001$ ). In Former SW (i.e., 15 individuals assessed in follow-up studies done 1.5 to 20 yrs

Address correspondence to Alain Reinberg, Unite de Chronobiologie, Fondation A. de Rothschild, 29 rue Manin, Paris, 75940 cedex 19, France. E-mail: areinberg@wanadoo.fr

after return to day work), both symptoms of intolerance and internal desynchronization were reduced or absent. The results suggest that non-tolerant SW are particularly sensitive to the internal desynchronization of their circadian time organization. (Author correspondence: [areinberg@wanadoo.fr](mailto:areinberg@wanadoo.fr))

**Keywords** Circadian period, Rhythm desynchronization, Hand grip strength, Oral temperature, Tolerance to shift work

## INTRODUCTION

Many shift workers (SW) find it difficult to adjust to night work. Nevertheless, some continue to work such shifts, while in others the intolerance and level of suffering are so intense that they cease being shift workers. These inter-individual differences raise questions about the basis of tolerance to shift work. The term tolerance is used here as it is applied in many life sciences, such as ergonomics (tolerance to effort), physiology (tolerance to anoxia), and pharmacology (tolerance to medications). Namely, it takes into account inter-individual differences (Andlauer et al., 1979; Axelsson et al., 2006; Reinberg et al., 1979a, 1979b, 1984a, 2007; Taylor et al., 1997). We have been particularly interested in individual differences in the time organization of circadian and other rhythms among human beings, and in this regard we previously documented the presence of allochronism (i.e., the desynchronization of various circadian rhythms in samples of healthy individuals without apparent medical consequence [Reinberg et al., 2007]). For many years, we and many occupational health physicians have been interested in finding indicators predictive of a worker's life-time tolerance to rotating SW schedules. However, the type of studies required to explore such indicators is extremely difficult to conduct. People would have to be studied intensively to gather longitudinal time series of meaningful variables both prior to their entrance into shift work as well as at discrete times during their shift-work career, with the data analyzed in relation to the worker's acute or chronic tolerance to SW based on medical and self-reported criteria. Unfortunately, the conduct of such studies is impractical. Thus, at present, the assessment of SW tolerance level is decided upon only after a period of time during which workers have been subjected to SW schedules.

Two, hopefully complementary, approaches have been used to explore this question. The conventional one makes use of a set of both clinical and chronobiologic criteria to decide, on an individual basis, whether or not a given SW is tolerant to night work. The other approach makes use of questionnaires to assess environmental, social, and psychological criteria (e.g., effects of supervisors, colleague support, team identity, job satisfaction, subjective well-being, and turnover intention), with the resulting database

used to build a group-phenomenon model to enable the SW a greater degree of control of the work environment and schedule to improve worker well-being and health (Fisher et al., 2006; Pisarski et al., 2006). The present study relies on a conventional biological approach (i.e., monitoring and analysis of individual time series gathered from workers over time with environmental factors and personal habits and behavior being carefully recorded).

In an earlier study of ours on SW (Reinberg et al., 1988a), we explored the relationship between the amplitude ( $A$ ) and period ( $\tau$ ) of circadian rhythms and SW tolerance. We reported poor SW tolerance is frequently associated with small circadian  $A$ s and prominent  $\tau$ s differing from 24.0 h, leading to an internal desynchronization of the circadian time organization. The study was carefully designed and involved non-selected SW, precise knowledge of the individual health status of the SW, longitudinal sampling of SW subjects, and application of power spectra analyses to time series data to accurately quantify the circadian  $A$ s and  $\tau$ s. The design of this study also makes it possible now for us to test, by a chronobiologic approach using these and other non-previously published time series data, the hypothesis that intolerance (in the form of dyschronism) to SW may result from the individual susceptibility of SW to internal desynchronization of circadian rhythms (Reinberg et al., 1984a, 1984b).

If the hypothesis is correct, one should expect that in a given population of non-selected SW that:

1. The mean age and mean SW duration will be longer in SW with good than poor tolerance and very poor tolerance (former SW discharged from night work). This also implies that the correlation between subject's age and the SW duration is stronger in tolerant than non-tolerant SW.
2. The number of desynchronized circadian rhythms will be greater in non-tolerant (those susceptible to desynchronization) than in tolerant SW when both are exposed to SW schedules.

Clinical experience shows that non-tolerant SW recover from their medical complaints and symptoms when they no longer engage in night work. Thus, in former SW who exhibited very poor tolerance to SW, the symptoms of SW intolerance and level of internal desynchronization should be expected to be reduced or absent after returning to day work exclusively. This observation adds another aspect to the hypothesis structure.

As there may be a certain level of internal desynchronization present in day workers and tolerant SW, we postulate that the increased level of

internal desynchronization that we hypothesize to occur in non-tolerant and poorly tolerant SW is induced and thus detected only upon exposure to SW schedules. This implies:

3. The identification of non-tolerance to SW can be pursued only while persons are exposed, rather than prior to exposure, to SW conditions.
4. Difference between SW tolerance and non-tolerance does not reside in rhythm-controlling mechanisms but rather in the entities that perceive exogenous time cue signals. Thus, the removal of the exogenous SW pressure, such as when intolerant SW move from SW to exclusively day-work schedules, should be expected to alleviate the inducing phenomenon and the resultant circadian rhythm internal desynchronization.

## **METHODS**

### **Subjects**

According to the French laws of Labor, all workers must undergo medical examination at least once per year by a qualified occupational health physician. Thus, we had access to up-to-date longitudinal medical/health records of workers. However, we selected SW for study not by criteria regarding health state, age, SW duration, industry, or SW rotation schedule, but by the sequence that the SW appeared for their mandatory medical examination. A total of 48 male Caucasian French SW volunteered for our field study; of these, two were left handed. All provided written consent according to the Helsinki ethical rules for human research and the standards of the journal (Touitou et al., 2006). They were not paid, but the cumulated times spent doing measurements at home, workplace, and elsewhere ( $\sim 10$  h/subject) were compensated by off-days from work. On average, the transportation time between work and home before and after work was  $\leq 30$  min. The home environment of all workers had quiet and comfortable conditions so as not to disturb sleep even when taken during the daytime hours (Rutenfranz et al., 1985). Subjects followed their spontaneous diet without restriction of quality and quantity. Meal timing was not taken into account in our analyses, as its synchronizing effect upon the examined variables has been found to be nil, even among SW (Reinberg et al., 1979a, 1979b).

### **Determination of SW Tolerance/Intolerance**

Intolerance to shift work was judged from both the personal experience and clinical examination of the subjects done twice per

year at the occasion of consultations with the occupational health physicians. The specific, traditional criteria of SW intolerance are complaints of:

- *Persisting fatigue (asthenia)*. Fatigue that persists for weeks or months is abnormal (Reinberg et al., 2007). Persistence means that non-work spans (i.e., weekends, off-days, and vacations) are ineffective for reversal or recovery from the fatigue. Although recent publications cite fatigue is a symptom of SW intolerance (Axelson, 2006; Fisher et al., 2006; Lammers-van der Holst et al., 2006; Pisarski et al., 2006; Van Dongen, 2006), to our surprise, reference to its *persistence* is lacking. Thus, we emphasize that the persistence of fatigue in SW is a major criterion of SW intolerance (Andlauer et al., 1979; Reinberg et al., 1979b, 1988a).
- *Persisting sleep alterations*. Sleep alterations in SW are trivial phenomena, but their persistence, again, is yet another major criterion of SW intolerance. Our studies involved the use of sleep logs to document alterations of difficulty in falling asleep, poor subjective sleep quality, frequent awakenings, and changed sleep duration.
- *Regular use of sleep-inducing medications*. Despite their ineffectiveness, the reliance upon sleep-inducing aids by self-medication and by prescribed medications of any type is almost a pathognomonic sign of SW intolerance (Reinberg et al., 2007).
- *Changes in behavior*. Unusual irritability, tantrum, verbal aggressivity, and sensitivity to noise are other signs of SW intolerance (Andlauer et al., 1979; Reinberg et al., 1979b, 1988a).
- *Digestive problems*. Symptoms such as indigestion, gastric ulcer, and altered bowel function are conventional signs of SW intolerance, although none of the studied subjects exhibited these.

After data gathering, three groups were considered for further analyses:

1. *Workers showing good SW tolerance*. Fourteen subjects who expressed neither complaints nor medical problems.
2. *Workers showing poor SW tolerance*. Nineteen subjects who expressed medical complaints for at least 1 yr. This group included 11 subjects with very poor SW tolerance. Their symptoms were so severe that the decision was made by medical personnel to transfer them from SW to day work as soon as such a position became available in the same industry. In some analyses, according to their specific aim, subjects with poor and very poor tolerance are pooled.
3. *Former SW with very poor tolerance*. Fifteen subjects previously discharged from night and switched to day work for at least 1.5 yr.

No subjects of this group expressed any complaints or medical problems at the time of their study.

Apart from SW with poor and very poor tolerance who took sleep medications, all subjects were medication-free at the time of study. Sleep medications that were available at the time of the study exerted little or no chronobiotic effect (Reinberg et al., 1988b). Alcohol and tobacco consumption was in rather low amounts, if any, and strictly forbidden at the workplace as stipulated by safety rules.

The shift workers came from three types of industries of different geographical locations: oil refinery (Rouen region of northwest France), and steel manufacturing and chemical engineering (both in the Lyon region in the central and east regions of France). Two types of SW rotations were concerned, the so-called “rapid rotation” (shift rotations every 2–4 days) and “weekly rotation” (shift rotations every 7 days). Four 8 h shifts were scheduled: a morning shift starting at 06:00 h, a day shift starting at 14:00 h, an evening shift starting at 22:00 h, and a night shift from 22:00 to 06:00 h. Off-days were scheduled after the night shift rotation. The distribution of tolerant, non-tolerant, and former SW was similar in all industries ( $\chi^2 = 1.98$ ,  $p > 0.05$ ) as well as the distribution of shift rotation type ( $\chi^2 = 2.56$ ,  $p > 0.05$ ). Moreover, the entry of subjects into the study by SW tolerance category was random as verified by sequential analysis methods (Armitage, 1960; Diem, 1963), which tested the distribution of subjects among the three groups. Details of subject entry into the studies can be found in Reinberg et al., 1988a.

### **Data Gathering**

Longitudinal data gathering took place for at least 15 consecutive days, including during one or two night shifts. A total of 30 subject studies was performed between February and September and 18 others between March and June. All subjects were trained in the use of the forms and instruments and properly standardized conditions. It took 5 to 10 min for a subject to perform the study measurements and tasks when at work, home, and/or elsewhere.

### **Study Variables/Measurements**

- Sleep-wake cycle was assessed by sleep log that gathered data on clock times of light-on and light-out plus subjective sleep quality, number of awakenings, etc.

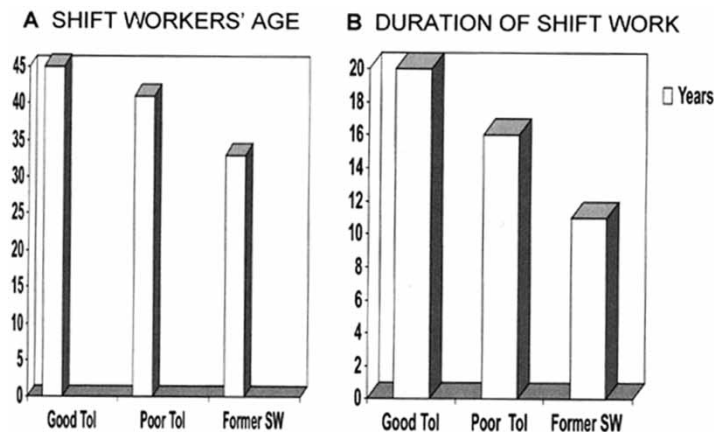
- Oral temperature circadian rhythm measured by a standard large-scale ( $0.05^{\circ}\text{C}$  precision) clinical thermometer; self-measurements were done daily, 4 to 5 times/24 h.
- Grip strength circadian rhythm of each hand was assessed by a Colin Gentile Dynamometer (Paris, France). Original files were retrieved and used. Data of the dominant hand were labeled “right hand” to facilitate reading. A hand-side-related difference in the grip strength circadian rhythm was previously reported by us (Reinberg et al., 1984b). Self-measurements were performed daily, 4 to 5 times/24 h.

### Statistical Analyses

Power spectra (De Prins & Malbecq, 1983), cosinor, t-test,  $\chi^2$  test, correlation coefficient ( $r$ ), and the Armitage method (Armitage, 1960; Diem, 1963) were applied to the data.

### RESULTS

As expected, the mean ( $\pm$ SEM) age of subjects showing good tolerance to SW was greater than that of those showing poor SW tolerance ( $44.9 \pm 2.1$  vs.  $40.1 \pm 2.6$  yrs,  $p < 0.001$ ), and the age of the latter was greater than that of former SW ( $33.4 \pm 1.7$  yrs,  $p < 0.001$ ) when they were discharged from night work due to very poor SW tolerance (see Figure 1A). Likewise, as expected, the duration of SW was longer in subjects having good tolerance than that of those having poor tolerance ( $19.9 \pm 2.2$  vs.  $15.7 \pm 2.2$  yr,  $p < 0.002$ ) and of former SW ( $10.7 \pm 1.4$  yrs,  $p = 0.0001$ ; see Figure 1B).

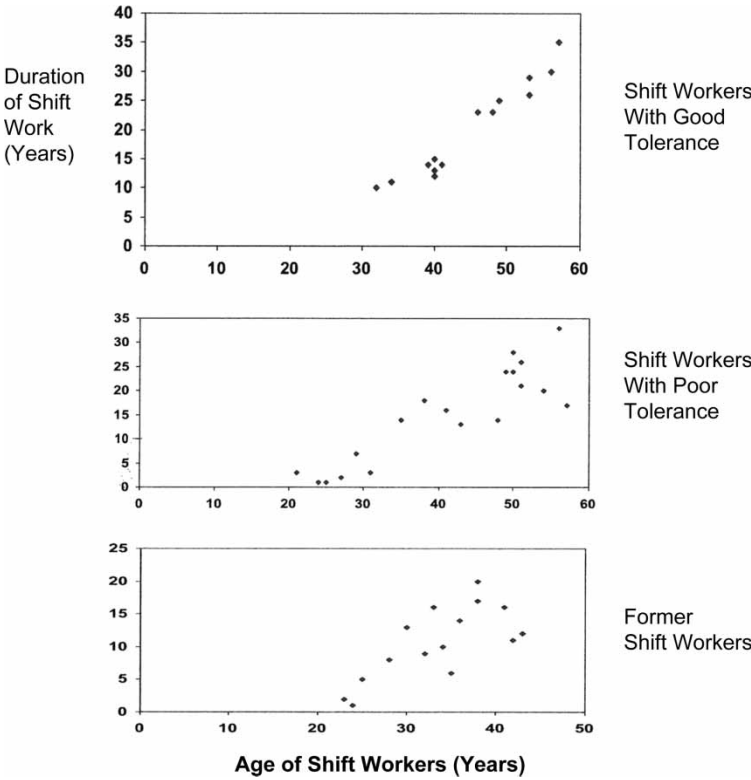


**FIGURE 1** (A) Shift workers' age. Ordinate: mean age  $\pm$  SEM (yr); abscissa: Good Tol = SW with good tolerance ( $44.9 \pm 2.1$  yr), Poor Tol = SW with poor tolerance ( $40.1 \pm 2.6$  yr), Former SW ( $33.4 \pm 1.7$  yr). (B) Duration (yrs) of shift work (mean  $\pm$  SEM); good SW tolerance ( $19.9 \pm 2.2$  yr); poor SW tolerance ( $15.7 \pm 2.2$  yr); former SW ( $10.7 \pm 1.4$  yr).



Correlation of Age and Duration of SW Career

To obtain a more complete understanding of SW intolerance and determine if there exists a threshold phenomenon in the SW duration of non-tolerant subjects, we assessed the relationship between age and duration of the SW career of non-tolerant subjects. Subject age and SW duration showed a very strong correlation ( $r = 0.97, p < 0.0001$ ) in tolerant SW. A weaker, yet statistically significant, correlation was also detected between the two variables in non-tolerant SW ( $r = 0.80, p < 0.001$ ) and also in former SW ( $r = 0.72, p < 0.01$ ). It is of interest (see Figure 2) that none of the non-tolerant subjects worked longer than 10 yr in SW. Indeed, the duration of shift work was  $<10$  yr in 6/19 non-tolerant SW (middle graph) and 6/15 former SW (bottom graph), but it was  $>10$  yr in all tolerant SW (15/15, top graph). A certain threshold effect of SW duration is apparent in the poor tolerant group; nonetheless, it seems that there are inter-individual differences in the SW duration in the subjects of both the non-tolerant and poorly tolerant SW groups.



**FIGURE 2** Correlation between subject age and SW duration. Top graph: tolerant SW ( $r = 0.97, p < .0001$ ); middle graph: poor SW tolerance ( $r = 0.80, p < .001$ ); bottom graph: former SW ( $r = 0.72, p < .01$ ).



### Sleep-Wake Cycle Tau

The mean period ( $\tau$ ) of the sleep-wake cycle of all 48 subjects, independent of SW tolerance status, was 24 h.

### Circadian $\tau$ of Oral Temperature Rhythm

More former and tolerant SW than non-tolerant SW evidenced oral temperature circadian rhythms with  $\tau = 24.0$  h (see Figure 3), the difference for the latter and former groups being statistically significant ( $\chi^2 = 26.5$ ,  $p = 0.0001$ ).

### Right-Hand Grip Strength

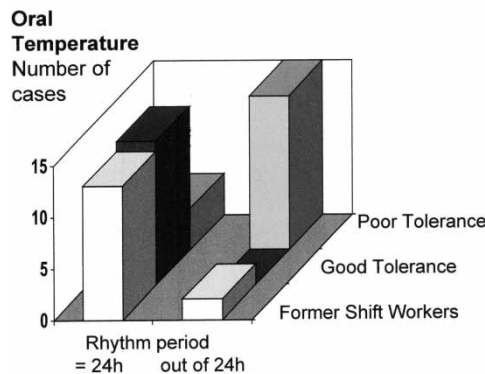
No difference ( $\chi^2 = 3.2$ ,  $p = 0.20$ ) was detected between former, non-tolerant, and tolerant SW in the number of subjects exhibiting rhythms with  $\tau = 24.0$  h (see Figure 4).

### Left-Hand Grip Strength

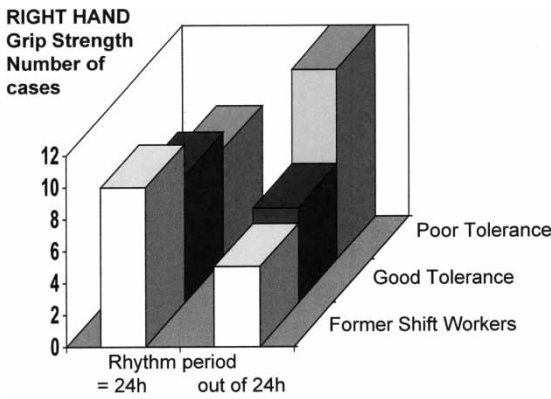
In contrast to the findings for the right hand, time series analyses of the left-hand grip strength data show (see Figure 5) more former and tolerant SW than non-tolerant SW show rhythms with  $\tau = 24.0$  h, the difference being statistically significant ( $\chi^2 = 19.2$ ,  $p < 0.0001$ ).

### Total Number of Circadian ( $\tau = 24.0$ h) Rhythms

A period ( $\tau$ ) of the oral temperature and/or hand grip strength circadian rhythm that differs from 24.0 h, given that the sleep-wake  $\tau = 24$  h, is

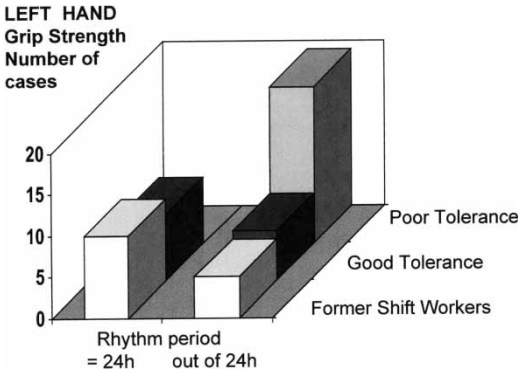


**FIGURE 3** Prominent period of oral temperature circadian rhythm. Left-side histograms: rhythm  $\tau = 24.0$  h; right-side histograms: rhythm  $\tau$  differing from 24.0 h. The difference in the distribution of  $\tau$  as a function of SW tolerance category is statistically significant ( $\chi^2 = 26.5$ ,  $p < 0.0001$ ).

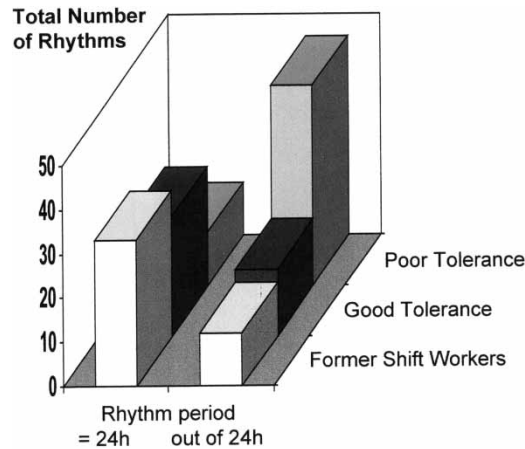


**FIGURE 4** Prominent period of right-hand grip strength circadian rhythm. Left-side histograms: rhythm  $\tau = 24.0$  h; right-side histograms: rhythm  $\tau$  differing from  $24.0$  h. No statistically significant difference in the distribution of  $\tau$  is validated, according to SW tolerance category.

indicative of internal desynchronization. As shown in Figure 6, desynchronization of the circadian time organization appears to be common in non-tolerant SW, the difference between this and the other two groups in the total number of rhythms with  $\tau = 24$  h being statistically significant ( $\chi^2 = 36.9, p < 0.0001$ ). Follow-up investigations of 15 former (very poorly tolerant) SW who were restudied 1.5–20 (median = 12) yr after transfer from shift to day work exclusively showed they were free of symptoms of SW intolerance. Figures 3 to 6 also show that the level of the internal desynchronization of the circadian rhythms in these subjects was reduced. This impression is validated by the fact that the number of rhythms for which  $\tau = 24$  does not differ between former and tolerant SW ( $\chi^2 = 0.33, p > 0.05$ ), while it does between former and non-tolerant SW ( $\chi^2 = 5.8, p < 0.05$ ).



**FIGURE 5** Prominent period of left-hand grip strength circadian rhythm. Left-side histograms: rhythm  $\tau = 24.0$  h; right-side histograms: rhythm  $\tau$  differing from  $24.0$  h. The difference in the distribution of  $\tau$  by SW tolerance category is statistically significant ( $\chi^2 = 19.2, p < 0.0001$ ).



**FIGURE 6** Total number of 24 h rhythms detected in oral temperature and right- and left-hand grip strength. Left-side histograms: rhythm  $\tau = 24.0$  h; right-side histograms: rhythm (differing from  $\tau = 24.0$  h). The difference in the number of  $\tau = 24.0$  h rhythms as a function of SW category is statistically significant ( $\chi^2 = 36.9, p < 0.0001$ ).

## DISCUSSION

Shift-work schedules are used in many industries. After several years of shift work, the employee is fully aware of the type of persisting fatigue, sleep alteration, and other symptoms that he is likely to experience. As stated above, some considered themselves capable of continuing a career in shift work. Those are referred to as SW with good tolerance. Those who do not tolerate shift-work are categorized as having poor tolerance and are frequently forced to give up their night-shift position and request or agree to be discharged (Andlauer et al., 1967, 1977; Landier & Vieux, 1976; Reinberg et al., 1979b; Vieux et al., 1963). In the industries for whom the subjects were working, the salary of the SW was not reduced when returning to day work in the same plant; thus, the decision was made without consideration of the financial impact upon the employee.

From a clinical point of view, it is known that the population of long-term tolerant SW is rather stable and homogeneous over time, as it results in a kind of self-selection. On the other hand, the population of non-tolerant shift workers is rather unstable due to the fact of its sustained elevated level of "evaporation" (Andlauer et al., 1977; Reinberg et al., 1979b). It should be emphasized that in many previous published studies, including those conducted by us (see appendix), subjects were not selected for their tolerance to shift work, and as stated earlier, such an a priori selection is not yet possible. In all of the studies, all subjects volunteered for the longitudinal data gathering. Time series were analyzed individually, and rhythm parameters were quantified. Only at this stage

did the investigators survey each subject's medical record to classify them as day workers, shift workers with good or poor tolerance, and former shift workers.

This important point is emphasized here because it relates to the question of the randomness of our sample. Meta analysis was performed on the results of eight different studies (see appendix) in which the period of the sleep/wake, oral temperature, and grip strength circadian rhythms were assessed longitudinally in tolerant, non-tolerant, and former SW, as well as day workers exposed to various environmental changes (see appendix). Our goal was to determine if the ratio of various categories of the subjects in each subgroup of the present study was similar to that of other studies as explored by the meta analysis. The meta analysis found tolerant SW made up 59.2% (74/125) of workers of the combined eight studies considered compared to 57.5% (14/33) workers in the present study. Taking the temperature rhythm as an example, the meta analysis showed the percentage ( $\pm$ SEM) of subjects with  $\tau = 24$  h was  $67.7 \pm 5.7\%$  in day workers,  $86.6 \pm 4.5\%$  in tolerant SW (vs. 100% in our study),  $39.5 \pm 8.1\%$  in non-tolerant SW (vs. 21% in our study), and 83–89% in former SW (vs. 86% in our study). Therefore, the distribution of the chosen subjects of the present study closely resembles that of the other investigations, thereby suggesting our sample is not a biased one.

In one of our previous investigations (Reinberg et al., 1988a), subjects were not selected, their respective entry in the study being randomized. Thus, the self-selection of non-tolerant SW is not likely to be an inherent bias. The design of that study also provided a means to test, by a chronobiologic approach, the hypothesis that intolerance to shift work (dyschronism) may result from one's susceptibility to internal desynchronization of circadian rhythms (Reinberg et al., 1984a, 1984b). Yet, at that time, the data were not analyzed to address this issue. Years later, while writing a review paper (Reinberg et al., 2007), we thought that it would be worthwhile to analyze these time series with a new aim in mind (i.e., to examine the validity of the hypothesis forwarded above). Some of the previously unpublished data were included.

If the hypothesis is correct, we may expect that, in a given random (non-selected) population of SW:

1. The mean age and mean shift-work duration will be longer in employees with a good tolerance than in ones with a poor tolerance and very poor tolerance to shift work (former SW discharged from night work). The above also implies the correlation between the worker's age and the duration of shift-work will be stronger in tolerant than non-tolerant SW.
2. The amount of desynchronized circadian rhythms will be greater in non-tolerant (those prone to desynchronize) than in tolerant workers when both are exposed to SW schedules.

Clinical experience shows that non-tolerant SW recover when they no longer engage in night work. Thus, in former SW with a very poor tolerance, both the symptoms of intolerance and the level of internal desynchronization will be reduced or completely resolved after returning to day work exclusively. This observation adds another aspect to the hypothesis structure:

3. As there can be a certain level of internal desynchronization in day workers and tolerant SW, we postulated that the increased level of internal desynchronization, which occurs in non-tolerant (and poorly tolerant) SW, can be induced and detected only upon exposure to shift work. This in turn implies that:
  - Identifying non-tolerance to SW schedules can be pursued only in SW conditions and not prior to entering such.
  - The difference between tolerance and non-tolerance to shift-work schedules does not reside in rhythm-controlling mechanisms, but in the entities that percept exogenous signals. Thus, removal of the exogenous pressure will cease the inducing phenomena.

With regard to the latter aspect, it should be cautioned that if the person remains on SW, persisting fatigue and sleep alterations may not be overcome (Andlauer et al., 1977; Ashkenazi et al., 1997; Reinberg et al., 1984; Vieux et al., 1963).

To the best of our knowledge, there has been no recent study that used a chronobiologic approach to better understand one's individual tolerance and intolerance to shift work. Tolerance is often not clearly characterized; in publications, references are made to fatigue and sleep alterations rather than to *persisting* fatigue and *persisting* sleep alterations (Axelson et al., 2006; Fischer et al., 2006; Lammers-van der Holst et al., 2006; Pisarski et al., 2006; Van Dongen, 2006). Thus, it is therefore difficult to compare results reported here with those reported by others, as we choose to use more restrictive criteria to define shift-work tolerance and intolerance. Moreover, in our study, a control group (Former SW) was included, enabling us also to examine the after-effects of exposure to shift-work schedules.

In their paper on ambulance personnel, Motohashi and Takano (1993) reported that the duration of shift work of workers with a good tolerance ( $n = 30$ ) was  $20.3 \pm 1.1$  yr (SEM), while for subjects with a poor tolerance ( $n = 12$ ), it was only  $7.7 \pm 3.4$  yr. Their results and ours are in good agreement.

With regard to studies considering that tolerance to shift work has exclusively an exogenous origin, our major concern relates to the fact that these authors (Fischer et al., 2006; Pisarski et al., 2006) did not take

into consideration the existence of inter-individual differences. As previously reported (Reinberg et al., 1988a), the symptoms of intolerance appear rather rapidly (within six months) and are related to neither age nor duration of shift work. The rather abrupt development of the symptoms of shift-work intolerance leads the worker, more or less rapidly, to consider that his night work has to be discontinued, whatever his age and seniority as a shift worker. In contrast, tolerant SW have no clinical reasons to leave the night shift. This is one of the major clinical differences between tolerant and non-tolerant SW.

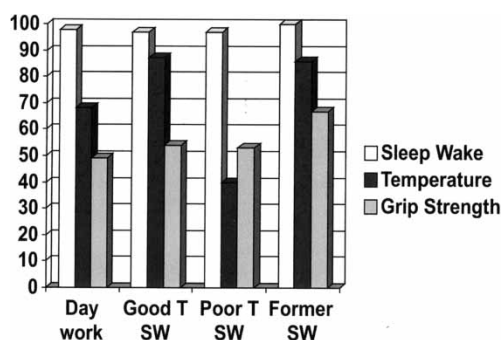
From this data re-analysis, it thus seems that internal desynchronization of the documented rhythms was associated with the SW intolerance. The fact that SW-related desynchronizations of circadian rhythms disappear when SW resume an exclusive day-work schedule has been reported previously by Reinberg et al. (1984a, 1984b, 1988a) as well as Chandrawanshi and Pati (2000) and Pati and Parganiha (2007). We may therefore consider that there is a relationship between the level of internal desynchronization actually evidenced by chronobiological means and the symptoms of SW intolerance, as both occur and persist during shift work and disappear or dissipate to the level seen when the employee is transferred to day work.

Presumably, several biological clock systems are involved in the reported desynchronization. The system controlling the sleep-wake rhythm seems to differ from the one controlling the body temperature rhythm, as earlier suggested by Aschoff and Wever (1981). Systems controlling right- and left-hand grip strength rhythms deserve to be taken into account. Their respective prominent period may differ from each other as well as from that of the temperature rhythm and obviously from that of the sleep-wake rhythm. In a previous study, we observed that there was a deviation of the prominent period of the body temperature rhythm from  $\tau = 24.0$  h. The same deviation was observed also in the period of the right-hand grip strength. We, therefore, conducted paired correlation tests between the prominent period of the rhythms of the investigated variables. The only detected correlation was between oral temperature and right-hand grip strength ( $r = 0.31, p < 0.05$ ). No correlation was found among the prominent  $\tau_s$  of the circadian rhythms of oral temperature, left-hand grip strength, peak expiratory flow (a measure of airway patency), or heart rate. The reanalysis of such data brings also to light the fact that the grip strength circadian rhythm of the right (dominant) hand is less prone to desynchronize than the left one (see Figures 4 and 5). Such a hand side-related difference was thereafter validated in rhythms of reaction times (Reinberg et al., 1997; Shub et al., 1997). These findings suggest the circadian clock system differs between the right and the left brain, with the possibility of coupling between the body temperature and right-hand grip rhythm.

An appendix regarding the meta analysis of internal desynchronization in SW is included, not only to address the problem of the randomness and representativeness of our worker sample, but also to provide estimates of

the number of workers showing allochronism and dyschronism of their circadian system (Reinberg et al., 2007), on the one hand, as well as tolerance to shift work, on the other. In Figure 7 and its legend, the results of the present study are included together with those of the meta analysis. This allows us to show that desynchronization of circadian rhythms can be rather common even in day workers without complaint (allochronism). It shows also that the incidence of circadian desynchronization is rather uncommon in former SW, a fact that suggests that dyschronism and related desynchronization are reversible in non-tolerant SWs.

In summary, this paper helps illustrate the relationship between, on the one hand, the clinical symptoms of SW intolerance recorded through the long-term follow-up (years and even decennia) and, on the other, internal desynchronization of the body's time organization, as determined by time series analyses of four different circadian rhythms documented longitudinally. The findings point to the fact that while the occurrence of internal desynchronization is greater among non-tolerant SW, this can only be documented when the susceptible worker is exposed to SW. Thus, at present, it is difficult a priori to determine a worker's susceptibility (i.e., non-tolerance) to shift work. The results of this study also raise the possibility that the biological entity that determines one's SW tolerance may reside in the individual inherited perception of the exogenous signals.



**FIGURE 7** Percent of the rhythms with prominent  $\tau = 24.0$  h. Figure includes data of the present study, which were added to those of the meta analysis cited in the Appendix. Day workers:  $n = 83$ ; Good T SW: shift workers with good tolerance ( $n = 79$ ); Poor T SW: shift workers with poor tolerance ( $n = 93$ ); Former SW: Former shift workers ( $n = 38$ ). Sleep-wake rhythms: percent of  $\tau = 24.0$  h; no statistically significant difference between groups; Temperature rhythm: desynchronization of the 24.0 h period found in  $32.3 \pm 5.1\%$  (SEM) of day workers (allochronism),  $13.4 \pm 4.5\%$  of tolerant SWs (i.e., allochronism),  $60.5 \pm 8.1\%$  of non-tolerant SW (i.e., dyschronism), and  $14.4 \pm 1.5\%$  of former SW (i.e., allochronism). Grip strength rhythm, right hand: desynchronization of the 24.0 h period found in  $51.2 \pm 8.0\%$  (SEM) of day workers (i.e., allochronism),  $46.6 \pm 2.0\%$  of tolerant SW (i.e., allochronism),  $45.3 \pm 8.8\%$  of non-tolerant SW (i.e., dyschronism), and  $25.6 \pm 8.5\%$  of former SW (i.e., allochronism).



APPENDIX

The percentage of rhythms with a prominent circadian period ( $\tau = 24.0$  h) of four variables (i.e., sleep wake, body temperature, grip strength of right and left hand), was documented longitudinally (for 7 to 14 days or more) in a set of investigations that included day workers and non-selected SW, studied in their habitual setting, with the SW sorted after the study into three subgroups of tolerant, non-tolerant, and former SW (see Figure 7). Data gathering and analyses (e.g., power spectra) as well as criteria used for categorizing SW tolerance were similar in all cases.

References for the Meta-analyzed Results

This entails eight different investigations (i.e., Ashkenazi et al., 1993; Chandrawanchi & Pati, 2000; Motohashi, 1990; Motohashi & Tanako, 1993; Motohashi et al., 1987, 1995; Reinberg, 2007, unpublished data; Reinberg et al., 1984a, 1984b, 1985, 1988a, 1989, 1994). With regard to desynchronization, the temperature circadian rhythm was taken as an example, as it was documented in all the quoted studies.

Usual Day Worker

Workers were exposed to their natural setting and usual life habits (Motohashi et al., 1987; Reinberg, 2007, unpublished data; Reinberg et al., 1985), including also environmental changes, such as intensive physical activities (Reinberg et al., 1984, 1989), placebo intake (Reinberg et al., 1994), and students with irregular retiring time (Motohashi, 1990). In total, there were seven studies (Motohashi, 1990; Motohashi et al., 1987; Reinberg, 2007, unpublished data; Reinberg et al., 1984, 1985, 1989, 1994) involving 83 workers (42 M & 41 F), median age 28 yrs, without medical complaint of any kind.

TABLE 1 Mean  $\pm$  SEM % Across the Studies of Workers Showing the Specified Rhythms with  $\tau = 24.0$  h

Sleep-wake (%)	Temperature (%)	Grip strength (%)	
		Right hand	Left hand
98.5 $\pm$ 1.3	67.7 $\pm$ 5.2	57.8 $\pm$ 8.9	54.5 $\pm$ 8.9

With reference to the temperature circadian rhythm, 32.3% of the workers were desynchronized (i.e., allochronism; t-test with  $p < 0.007$ ).

### Tolerant Male SW

Different industries and different types of shift rotation: five studies (Ashkenazi et al., 1993; Chandrawanchi & Pati, 2000; Motohashi & Tanako, 1993; Reinberg et al., 1984a, 1985) involving a total of 74 SW tolerant out of 125 workers.

**TABLE 2** Mean  $\pm$  SEM % Across the Studies of Workers Showing the Specified Rhythms with = 24.0 h

Sleep-wake (%)	Temperature (%)	Grip strength (%)	
		Right hand	Left hand
96.9 $\pm$ 1.7	83.3 $\pm$ 4.5	53.4 $\pm$ 2.0	—

With reference to the temperature circadian rhythm, 16.7% of the workers were desynchronized (i.e., allochronism; t-test with  $p < 0.01$ ).

### Non-tolerant Male SW

Different industries and different types of shift rotations; four studies (Ashkenazi et al., 1993; Chandrawanchi & Pati, 2000; Motohashi & Tanako, 1993; Reinberg et al., 1984a, 1985) involving 51 non-tolerant out of 125 workers.

**TABLE 3** Mean  $\pm$  SEM % Across the Studies of Workers Showing the Specified Rhythms with = 24.0 h

Sleep-wake (%)	Temperature (%)	Grip strength (%)	
		Right hand	Left hand
96.9 $\pm$ 1.0	44.2 $\pm$ 9.1	54.7 $\pm$ 9.0	36 $\pm$ 10.4

With reference to the temperature circadian rhythm, 55.8% of the workers were desynchronized with symptoms of intolerance (i.e., dyschronism; t-test with  $p < 0.0001$ ).

### Former SW

Workers (n = 24) who were discharged from night work due to poor tolerance, exclusively performing day work in the same industry for at

least 1.5 yr. Many of them were studied twice (see Chandrawanchi & Pati, 2000; Reinberg et al., 1984a; 1988a).

**TABLE 4** Mean  $\pm$  SEM % Across the Studies of Workers Showing the Specified Rhythms with = 24.0 h

Sleep-wake (%)	Temperature (%)	Grip strength (%)	
		Right hand	Left hand
100 $\pm$ 0	83 and 89	—	—

With reference to the temperature circadian rhythm, 11–17% of the workers were symptom-free and desynchronized.

ACKNOWLEDGMENT

The study was been performed with the help of the Therese Tremel-Pontremoli donation for chronobiologic research at the Fondation Adolphe de Rothschild, Paris. We express our special thanks to Erhard Haus and Michael Smolensky for their most pertinent and helpful comments.

REFERENCES

Andlauer P, Metz B. (1967). Le travail en équipes alternantes. In: Scherrer J (ed.) *Physiologie du travail (Ergonomie)*. Paris: Masson, pp. 272–281.

Andlauer P, Carpentier J, Cazamian P. (1977). *Ergonomie du travail de nuit et des horaires alternants*. Education Permanente, Université de Paris. Paris: Editions Cujas, 247 pp.

Andlauer P, Reinberg A, Fourre L, Battle W, Duverneuil G. (1979). Amplitude of the oral temperature and tolerance to shift work. *J. Physiol. (Paris)* 75:507–512.

Armitage P. (1960). *Sequential Medical Trials*. Oxford: Oxford University Press.

Aschoff J, Wever R. (1981). The circadian system of man. In Ashoff J (ed.) *Handbook on Behavioral Neurobiology: Biological Rhythms*. London: Plenum, pp. 311–331.

Ashkenazi IE, Reinberg A, Bickova-Rocher A, Ticher A. (1993). The genetic background of individual variations of circadian rhythm period in healthy human adults. *Am. J. Hum. Genet.* 52:1250–1259.

Ashkenazi IE, Reinberg AE, Motohashi Y. (1997). Interindividual differences in the flexibility of human temporal organization: Pertinence to jet lag and shift work. *Chronobiol. Int.* 14:99–113.

Axelsson J, Lowden A, Kecklund G. (2006). Recovery after shift work: Relation to coronary risk factor in women. *Chronobiol. Int.* 23:1115–1124.

Chandrawanshi A, Pati AK. (2000). Does externally desynchronized circadian rhythms could be resynchronized in shift workers. *Biol. Rhythm Res.* 31:160–176.

De Prins J, Malbecq W. (1983). Analyses spectrales pour données non équidistantes. *Bull. Acad. Sci. Belg. série 5e.* 69:287–294.

Diem K. (1963). *Documenta Geigy: Scientific Tables*, 5th edn. Basel: JR Geigy, SA, 199 pp.

Fischer FM, da Silva Borges FN, Rotenberg L, de Oliveira Lattore MRD, Soares NS, Santa Rosa PLF, Teixeira LR, Nagai R, Steluti J, Landsbergis P. (2006). Work ability of health care shift worker: What matters. *Chronobiol. Int.* 23:1165–1179.

- Lammers-van der Holst HM, Van Dongen HPA, Kerkhof GA. (2006). Are individuals' nighttime sleep characteristics prior to shift work exposure predictive for parameters of daytime sleep after commencing shift work? *Chronobiol. Int.* 23:1217–1227.
- Landier H, Vieux N. (1976). *Le travail posté en question*. Paris: Editions du Cerf, 189 pp.
- Motohashi Y. (1990). Desynchronization of oral temperature and grip strength circadian rhythms in healthy subjects with irregular sleep-wake behavior. In Hayes DK, Pauly JE, Reiter RJ (eds.) *Chronobiology in Clinical Medicine, General Biology and Agriculture*. New York: Wiley-Liss, pp. 57–63.
- Motohashi Y, Takano T. (1993). Effects of 24-hour shift work with nighttime napping on circadian rhythm characteristics in ambulance personnel. *Chronobiol. Int.* 10:461–470.
- Motohashi Y, Reinberg A, Lévi F, Nougier J, Benoit O, Forest J, Bourdeleau P. (1987). Axillary temperature a circadian marker rhythm for shift workers. *Ergonomics* 30:1235–1247.
- Motohashi Y, Reinberg A, Ashkenazi IE, Bickova-Rocher A. (1995). Genetic aspects of circadian dyschronism: Comparison between Asiatic-Japanese and Caucasian-French populations. *Chronobiol. Int.* 12:324–332.
- Pati AK, Parganiha A. (2007). Shift work: Circadian rhythm disruption and beyond. *Proc. Ind. Natl. Sci. Acad.* B71–5/6:229–255.
- Pisarski A, Brook C, Bohle P, Gallois C, Watson B, Winch S. (2006). Extending a model of work tolerance. *Chronobiol. Int.* 23:1363–1377.
- Reinberg A, Migraine C, Apfelbaum M, Grigant L, Ghata J, Vieux N, Laporte A, Nicolai A. (1979a). Circadian and ultradian rhythms in the feeding behavior and nutrient intakes of oil refinery operators with shift work every 3–4 days. *Diabet. Metabol. (Paris)* 3:33–41.
- Reinberg A, Vieux N, Andlauer P, Guillet P, Laporte A, Nicolai A. (1979b). Oral temperature, circadian amplitude and tolerance to shift work. *Chronobiologia Suppl.* 1:77–85.
- Reinberg A, Andlauer P, Bourdeleau P, Lévi F, Bickova-Rocher A. (1984a). Rythmes circadiens de la force des mains droites et gauches: Désynchronisation chez certains travailleurs postés. *Comptes Rendus Acad. Sci. (Paris)* 299:633–636.
- Reinberg A, Andlauer P, De Prins J, Malbecq W, Vieux N, Bourdeleau P. (1984b). Desynchronization of the oral temperature circadian rhythm and tolerance to shift work. *Nature* 308:272–274.
- Reinberg A, Motohashi Y, Bourdeleau P, Andlauer P, Lévi F, Bickova-Rocher A. (1988a). Alteration of period and amplitude of circadian rhythm in shift workers with special reference to temperature, right and left hand grip strength. *Eur. J. Appl. Physiol.* 57:15–25.
- Reinberg A, Smolensky M, Labrecque G. (1988b). The hunting of a wonder pill for resetting all biological clocks. *Ann. Rev. Chronopharmacol.* 4:171–208.
- Reinberg A, Bickova-Rocher A, Nougier J, Gorceix A, Mechkouri M, Touitou Y, Ashkenazi IE. (1997). Circadian rhythm period in reaction time to light signals: Difference between right- and left-hand side. *Cogn. Brain Res.* 6:135–140.
- Reinberg A, Ashkenazi I, Smolensky MH. (2007). Is desynchronization a sign of sickness? Echronism, allochronism, dyschronism. *Chronobiol. Int.* 24:553–588.
- Rutenfranz J, Haider M, Koller M. (1985). Occupational health measures for nightworkers and shiftworkers. In Folkard S, Monk TH (eds.) *Hours of Work. Temporal Factors in Work Scheduling*. Chichester: John Wiley & Sons, pp. 199–210.
- Shub Y, Ashkenazi IE, Reinberg A. (1997). Difference between left- and right-hand reaction time: Indications of shifts in strategies of human brain activities. *Cogn. Brain Res.* 6:141–146.
- Taylor E, Briner RB, Folkard S. (1997). Models of shift work and health: An examination of the influence of stress on shift work theory. *Hum. Factors* 39:67–82.
- Touitou Y, Portaluppi F, Smolensky MH. (2006). Ethics, standards, and procedures of animal and human chronobiology research. *Chronobiol. Int.* 23:1083–1096.
- Van Dongen HPA. (2006). Shift work and inter-individual differences in sleep and sleepiness. *Chronobiol. Int.* 23:1139–1148.
- Vieux N, Carré D, Demones P. (1963). Le travail de quart continu dans une raffinerie de pétrole. *Arch. Mal Prof.* 24:139–143.