Circadian rhythms of micturition during jet lag

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ABSTRACT

Micturition behavior follows regular day/night fluctuations, and unwanted increase in micturition could occur during night in jet lag condition. To clarify the effect of jet lag on micturition behavior, we simultaneously detected circadian micturition patterns and locomotor activity rhythms of mice under experimental jet lag conditions, by applying the improved automated Voided Stain on Paper (aVSOP) method. When wild-type (WT) mice were phase-advanced for 8 hours, day-night variation of micturition was disrupted suddenly, and this irregular daily micturition continued until 8 days, although their activity rhythms entrained gradually day by day until 8 days. We also examined how jet lag induced changes of micturition in *Per*-null mice lacking *Per1*, *Per2* and *Per3* genes, whose endogenous clock is completely disrupted. We found both micturition and locomotor activity of *Per*-null mice promptly entrained to the new LD cycle. These findings suggest that the irregular micturition during jet lag is caused along with the gradual shift of the endogenous clock, and paradoxically, jet lag-associated abnormality was absent when endogenous circadian oscillations were genetically disrupted.

In mammals, most organs have an ability to generate circadian rhythms that coordinate various physiological phenomena (4, 19). This basic clock mechanism, composed of clock genes interlocked in transcription-translation feedback loops in which *Period* genes (*Per1*, *Per2* and *Per3*) play a central role, exists in virtually all cells in the body (5, 11, 13). The circadian rhythms in all cells are synchronized by the master clock in the hypothalamic suprachiasmatic nucleus (SCN). Through this synchronization, the SCN drives rhythms in physiologically relevant phenomena such as body temperature and blood pressure (10, 15, 25). The SCN also engages in entraining rhythms to the environmental light-dark

cycles, so that endogenous circadian rhythms are normally entrained to the ambient solar time. However, when traveling across multiple time zones, the internal clock shows temporal desynchronization with the external solar time (6, 12). This desynchronization evokes jet lag symptoms, including sleep disturbances and gastrointestinal distress (3, 14), although their precise pathophysiology has been unexplored.

As an important physiological function, micturition behavior also shows circadian rhythms. The increase of urine production in the kidneys and the decrease of storage capacity of the urinary bladder urge micturition behavior. In a healthy person, urine production is reduced and urine storage capacity is increased at night for a sound sleep (7, 21, 23). Nocturnal enuresis in children and nocturia in the elderly are speculated to be caused by a mismatch between urine production rate in the kidneys and storage capacity of the urinary bladder (20, 22). In

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jet lag, frequent micturition often disturbs sleep, since the clock cannot be instantly reset to local time but continues to oscillate autonomously on its own time for several cycles. However, until now, implication of circadian rhythm in day/night micturition behaviors in jet lag, has not been established.

Since mice are nocturnal, micturition occurs more often at night, but its accurate frequency and volume have not been clarified for a long time because of their minute urine volume voided per micturition (18, 24). Recently, Negoro *et al.* (8) succeeded to record circadian micturition rhythms in free-moving mice by the automated Voided Stain on Paper (aVSOP) method, which can accurately record micturition of mice. In the present study, we examined the disrupted micturition rhythms during jet-lag by an improved aVSOP method, which enables to record micturition rhythms and behavioral rhythms simultaneously. We examined the change of the micturition rhythm not only in wild-type (WT) mice, but also in *Per*-null (*Per1*, *Per2*, and *Per3* knockout) mice whose clock oscillation is disrupted.

MATERIALS AND METHODS

Animals. Male *Per*-null mice (*Per1*^{-/-}, *Per2*^{-/-}, and *Per3*^{-/-}) (1) and wild-type Balb/c mice (WT) aged 10–16 weeks were used. We generated *Per*-null mice by crossing *Per1*-knockout (1), *Per2*^{Brdm1}-mutant (29), and *Per3*-knockout (16) mice, on the BALB/c strain with backcrosses for at least 10 generations (1). Mice were housed individually in wire net cages (5.0 mm × 5.0 mm square net with wire diameter 0.7 mm) with *ad libitum* access to food and water. The animals were treated in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee.

Measurement of behavioral and micturition rhythms. *Per*-null and WT mice were entrained to a 12-h-light (~200 lux fluorescent light)/12-h-dark (LD) cycle at least two weeks to entrain the circadian clock of the mice to the ambient LD cycle, and then LD cycles were 8-h phase-advanced. Locomotor activity was recorded in 5-min bins with passive infrared sensor, and the data obtained were analysed with Clocklab software (Actimetrics) developed on MatLab (Mathworks). Micturition was recorded by using the aVSOP method. Rolled laminated filter paper, pre-treated to turn the edge of urine stains deep purple, was wound up at a speed of 5 cm per hour under a water-repellent wire lattice. Each urine stain was traced, scanned and quantified by Image J 1.42

software to convert micturition volume using the formula of standard curve. Both locomotor activity and micturition rhythms were measured for 5 days under LD condition, followed by 14 days after the phase advance. Total urine volume per hour was estimated by dividing the volume by the time interval between the given and preceding voiding (filling time), when the filling time was more than 1 h (21). Compared to our previous report using the aVSOP system (8), the dimensions of the mouse support wire lattice were optimized, reducing the size of holes to improve ambulatory stress.

RESULTS

Simultaneous measurement of locomotor activity and micturition rhythms

In our improved aVSOP system (Fig. 1A), urine at each micturition was traced on the moving filter paper as dots (Fig. 1B). To generate a standard formula to show the correlation of liquid volume and stained area of a dot on filter paper, normal saline ranging from 10 to 800 μ L was poured on a filter paper (Fig. 1C). We scanned and quantified these saline stains, and found a clear linear correlation between poured volume and the stained area ranging from 10–800 μ L.

When animals were housed in LD conditions, WT mice showed a higher locomotor activity in the night time than in the day time (Fig. 2A). Similar to the locomotor activity, the micturition frequency of WT mice was higher in night time (6.25 ± 0.78 times/12 h, $n = 5$) than in day time (0.542 ± 0.152 times/12 h, $n = 5$) (Fig. 2B). Similarly, the total void volume per hour also showed an increase in night time (Fig. 2B, lower panel).

In *Per*-null mice whose circadian clock is completely disrupted, locomotor activity was high in the night time, and low in the day time as reported previously (2). Similarly, the micturition frequency and void volume of micturition of *Per*-null mice showed temporal variation under LD cycles (Figs. 3A and 3B). This “rhythm” is not endogenous, and the diurnal difference of locomotor activity and micturition is caused by the inhibition of locomotor activity by external light.

Locomotor activity and micturition rhythms in jet lag condition

Next, we examined the locomotor activity (Figs. 2A and 3A) and the urine volume voided per micturition (Figs. 2B and 3B) simultaneously, under experimental jet lag conditions in WT and *Per*-null mice

Fig. 1 Simultaneous measurement of locomotor activity and micturition rhythms. **(A)** A diagram showing the simultaneous measurement of locomotor activity by the infrared sensor and micturition by aVSOP method. **(B)** Representative urine spots on paper are shown. Motor drives paper at speed of 5 cm/h (Bar = 1 hour). Each urine dot on paper was traced, scanned and quantified by Image J 1.42 software. **(C)** A graph showing a standard curve constructed from 10 to 800 μL of normal saline and their corresponding stained areas ($n = 5$ for each volume).

(Fig. 4A). In WT mice, urination showed temporal desynchronization from environmental LD cycles for several days after jet-lag similar to locomotor activity rhythms (Figs. 2A and 4A). In contrast, urination of *Per*-null mice tended to abruptly entrain to the environmental LD cycles, as observed for their locomotor activity rhythms (Figs. 3A and 4A). However, because micturition occurred inconsistently, often with several hours intervals, we found it difficult to quantitatively measure the re-entrainment speed of WT and mutant mice based on the observed changes in voided volume and micturition timing (Fig. 4A).

To overcome this problem, we quantified the estimated urine production per hour (Fig. 4B) by dividing the void volume by the time interval between the given and preceding voidings (21); the data were also displayed in double-plot format for the comparison with that of locomotor activity rhythm (Fig. 5A). In WT mice, an 8-hour phase advance evoked a gradual shift of locomotor activity rhythms and micturition rhythms, and it took 6 to 8 days for complete re-entrainment to the new LD schedule. In contrast, *Per*-null mice showed almost immediate re-entrainment with only 2 to 4 days of transition (Figs. 5A and 5B). In both genotypes, there was no apparent

discrepancy between re-entrainment of micturition and of locomotor activity (Fig. 5B).

DISCUSSION

The present study demonstrated that it takes about a week for complete entrainment of micturition, as well as locomotor activity, to the 8-h phase advance of LD cycles. Since the temporal desynchronization with the external solar time under this jet-lag schedule is similar to transmeridian flights, mice can be used as a model for analyzing disrupted rhythms, although the day-night change is inverted because mice are nocturnal.

The production of urine by the kidney, and the storage of urine in the urinary bladder are key steps in micturition (27). Importantly, the regulation of storage or voiding of the urine depends on central as well as peripheral autonomic and somatic neural pathways, which receive higher-level regulatory input from various brain and spinal cord nuclei (28). Since clock genes are expressed in virtually most cells of the body (14, 22), the biological clock may intrinsically regulate micturition by acting on any, if not all, of these control centers. Moreover, these structures indirectly receive circadian input from the

Fig. 2 Locomotor activity and micturition rhythms in WT mice. **(A)** Representative double plotted actogram of WT mice in LD condition, which is later subjected to an 8-hour phase advance in LD cycles. The advance was conducted on day 6. Top bars correspond with initial LD setting and shades indicate dark phases. **(B)** Representative charts of urine volume voided per micturition (upper panel) and urine voided per hour (lower panel) of WT mice under LD cycles for 3 days. Hours after the onset of light phase of the first day are indicated on white/black bars (upper panel) or white/gray backgrounds (lower panel) showing lights on and off, respectively.

master clock in the hypothalamic suprachiasmatic nucleus (SCN), which is the master clock in the body (3). Potentially, the SCN can synchronize clock gene oscillations in all structures related to the control of micturition.

During jet lag, the SCN temporarily loses the ability to generate rhythms and to synchronize body clocks, and it takes about a week for the rhythms to recover (23). During the adjustment period, the SCN will gradually phase shift while recovering its rhythm-integration ability. This is reflected at the behavioral level, progressively shifting to the new environmental cycles. Together with these rhythms in locomotor activity, micturition rhythms will also phase shift gradually.

Fig. 3 Locomotor activity and micturition rhythms in *Per*-null mice. **(A)** Representative double plotted actogram of *Per*-null mice in LD condition, which is later subjected to an 8-hour phase advance in LD cycles. The advance was conducted on day 6. Top bars correspond with initial LD setting and shades indicate dark phases. **(B)** Representative charts of urine volume voided per micturition of *Per*-null mice under LD cycles for 3 days. Hours after the onset of light phase of the first day are indicated on white/black bars (upper panel) or white/gray backgrounds (lower panel) showing lights on and off, respectively. Note that urination occurs mostly in the night time in this arrhythmic genotype.

During these processes, timing of urine production will be affected by the shift of drinking behavior. In addition, primitive urine production and water reabsorption in the kidneys (17), which play important role for regulating circadian urine production, may also be affected by jet lag. The urinary bladder also shows diurnal change of capacity to hold urine (9). We previously demonstrated that clock genes in bladder smooth muscle cells play a very important role in the formation of micturition rhythms by regulating the transcription of the gap junction protein Connexin 43 (Cx43), rising in the active phase (night time in the mouse) together with an increase of gap-mediated signals in smooth muscle cells (7). As Cx43 is only weakly expressed in the resting phase

Fig. 4 Micturition rhythms in jet-lag conditions in WT and *Per*-null mice. **(A)** Representative charts of urine volume voided per micturition (orange diamonds) and the locomotor activity per hour (black line) of WT (top) and *Per*-null (bottom) mice in jet-lag conditions. **(B)** Representative charts of total urine volume voided per hour of WT (top) and *Per*-null (bottom) mice in jet-lag conditions.

(inactive phase: day time in mice), cholinergic effects are suppressed, and bladder capacity is enlarged (7). The role of Cx43 in bladder smooth muscle in jet lag-induced micturition changes is a potential route for further investigations.

Here, we found that the time taken to adapt to the new LD cycle in the micturition rhythms is similar to that of locomotor activity rhythms. Since it is speculated that adaptation of the locomotor activity to the new LD cycle is regulated by the SCN (26), similar regulatory mechanism of micturition rhythm should be tested in future studies.

In both micturition and locomotor activity, *Per*-null mice entrained more promptly to the new LD cycles than WT mice under experimental jet-lag conditions. *Per*-null mice did not show circadian rhythms in constant dark condition (2) since these

mice lack the ability to produce endogenous rhythms. Taken together, it seems likely that jet lag symptoms on locomotor activity and micturition are caused due to the stability of the endogenous clock, and paradoxically, an impaired circadian clock may be desirable to alleviate jet lag symptoms.

Clinically, circadian clock may play an important role for maintaining day and night micturition cycle in healthy persons. The present study may indicate that micturition cycle could be disturbed by jet lag and takes time for complete recovery. This point has not been addressed in scientific literatures, but may be worth considering for assessing micturition-associated bother in frequent flyers and time-shift workers.

Fig. 5 Re-entrainment of locomotor activity and micturition rhythms after jet lag. **(A)** Representative double-plotted actogram (top) and hourly urine production data (bottom) of WT and *Per*-null mice subjected to an 8-hour phase advance in LD cycles. **(B)** The days required for reentrainment of locomotor activity and micturition in WT and *Per*-null mice. Means ± s.e.m., $n = 7$ (both genotypes); *** $P < 0.001$, n.s. $P = 0.3559$ and 0.3559 , paired Student's *t*-test.

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