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EFFECT OF RESTRAINT STRESS ON ALTERNATION OF CIRCADIAN BLADDER FUNCTION TO INDUCE NOCTURIA

Hypothesis / aims of study

The pathophysiologies of nocturia are multifactorial and complex. Their etiologies remain unclear in a large number of patients. Previously, we reported that urination behavior in mice showed circadian rhythm which is regulated by clock genes and the abnormalities of clock genes might cause nocturia because of the loss of circadian bladder function (1). On the other hand, it has been reported that anxiety or post-traumatic-stress-disorder could increase nocturnal voiding (2). Furthermore, some types of continuous acute stress such as restraint stress (RS) caused disruption of the circadian rhythm only in peripheral organs, without any effect on central nervous system in mice (3). In the present study, to reveal effect of restraint stress on alternation of circadian bladder function, we investigated urination behaviour and expression rhythm of clock genes in the mice bladder after restraint stress.

Study design, materials and methods

Male C57BL/6 mice aged 8 -12 weeks were bred under 12 h light/dark conditions for 2 week. The light period started from 6 am [Zeitgeber time (ZT) 0)]. Mice were individually placed into the metabolic cages, and were subjected to RS for 2 hrs from ZT4 to ZT6 by enfolding using metal mesh sized 12cm×12cm after the aesthesia of sevoflurane. RS was applied for 5 days (from RS1 to RS5) and the urination behavior for 24 hrs was continuously recorded from baseline to RS5. In control, urination behavior was recorded without any RS in metabolic cages for 6 days (baseline and from Day1 to Day5). The following parameters were measured both in control and RS mice, respectively: water intake volume (WIV), voiding frequency (VF), urine volume (UV) and urine volume/void (Uvol/v). Urination during the light period was counted as a nocturnal voiding.

In the measurement of gene expression rhythm in clock genes, *Period2*^{luciferase} knock-in mice (Per2::luc) were used. *Per2* is one of the clock genes, which act as a negative transcriptional factor for clock-controlled-genes. The bladder was removed from Per2::luc mice at ZT10, which is the peak time of *Per2* expression. Then the bladder was placed into the dish type lumino-meter immediately. The bioluminescence of luciferin-luciferase reaction from removed bladder was measured for 3 days and *Per2* expression rhythm was compared between control and RS mice. 15μM Forskolin was used in order to confirm the viability of bladder tissue at the end of measurement. Data were analysed using Mann-Whitney's *U*-test, and a one-way ANOVA with Bonferroni test.

Results

The body weight (BW), WIV and UV for 24 hrs showed no difference between control and RS mice. VF in the dark on Day 5 was significantly higher than baseline in control mice. However, RS mice did not show any difference in VF in the dark. In contrast, VF in the light did not show any difference through measurement in control mice. However, VF in the light increased significantly higher after RS compared to baseline (Fig. 1). Uvol/v in mice has the circadian rhythm, which was higher in the light than in the dark. This rhythm was observed only in baseline and disappeared after RS in RS mice. Moreover, a decrease of Uvol/v was not observed in the dark in RS mice. However, Uvol/v in the light was significantly lower at Day1 and Day 3 compared to baseline in RS (Fig. 2).

Per2 expression showed circadian rhythm in control mice. RS mice also showed rhythmic expression in Per2. However, the oscillation cycle was shorter in RS mice than control mice (Fig. 3).

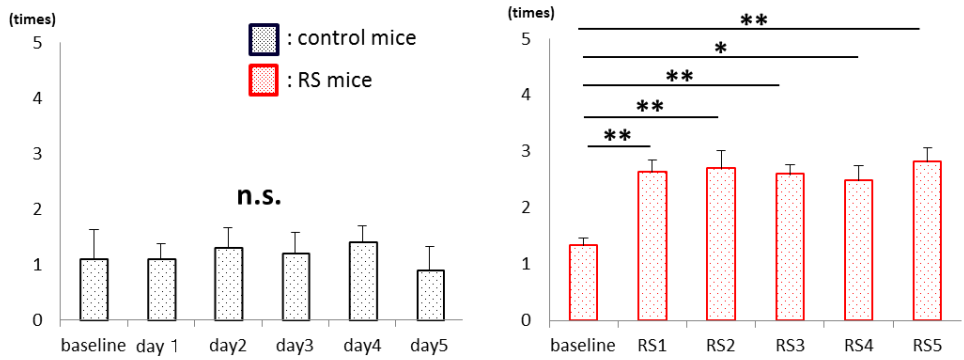
Interpretation of results

The physical stress in the sleep phase could alter the circadian rhythm of clock genes in the bladder. This result indicated that RS induce nocturia through the changes of circadian bladder function.

Concluding message

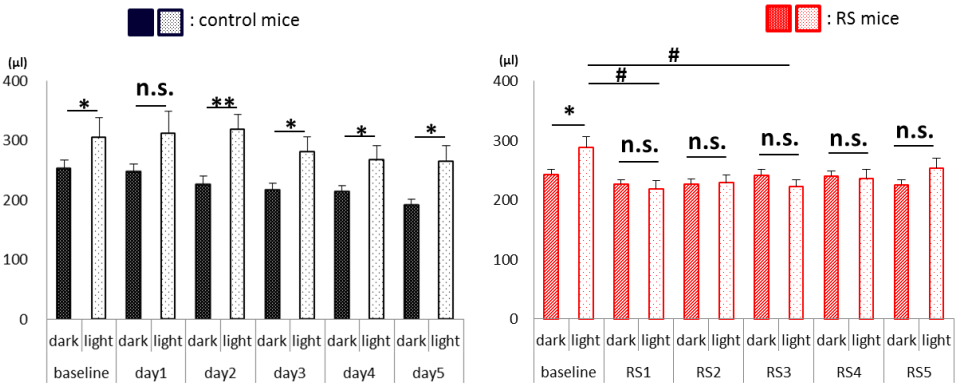
Abnormalities of circadian rhythm in the urinary bladder caused by physical stress are one of important factors in changes of urination pattern, particularly nocturia.

Figure 1. Voiding frequency in the light phase



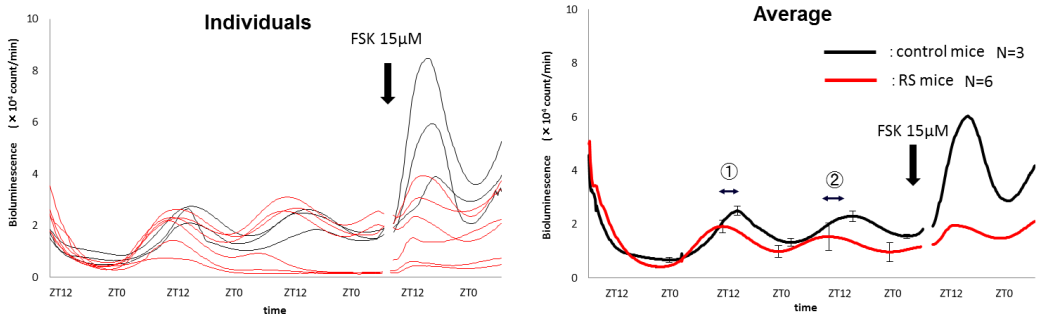
* P<0.05, ** P<0.01, One-way ANOVA with Bonferroni test

Figure 2. Urine volume/void



* P < 0.05, ** P<0.01, Man Whitney's *U*-test. # P<0.05, One-way ANOVA with Bonferroni test.

Figure 3. Monitoring of Per2::Luc bioluminescence.



Deviations of the peak time in *Per2* expression

①: - 2 h 40 min ②: - 4 h 10 min

References

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Disclosures

Funding: I do not have any affiliations to disclose. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** mouse **Ethics Committee:** the Physiological Society of Japan and the policies of the Institutional Animal Care and Use Committee of the University of Yamanashi