

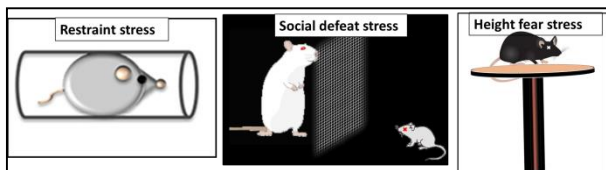


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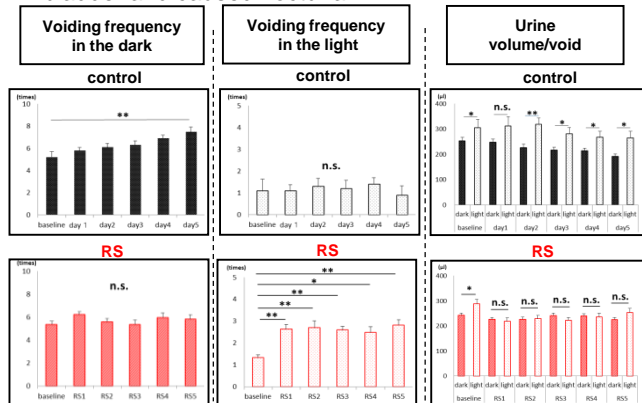
Introduction

- We reported that the sensation of bladder fullness had circadian rhythm through gene expression rhythm of mechano-sensors such as Piezo1 and TRPV4 under the regulation by clock genes in the mouse bladder urothelium (1). Then, Urination behavior showed circadian rhythm, and the abnormalities of clock genes might cause nocturia because of the loss of circadian bladder function such as the sensation of bladder fullness.
- Circadian regulations of clock genes are altered by some types of intermittent stress, such as restraint stress (RS), only in peripheral organs in mice (2).



Some types of stress, indicated in the upper illustrations, altered the circadian gene expression only in the peripheral tissue without any effects on to CNS in mice

- At ICS2017, we have reported that intermittent RS induced nocturia in mice and hypothesized that abnormal circadian clock induced by RS could occur in the mice bladder and caused nocturia.



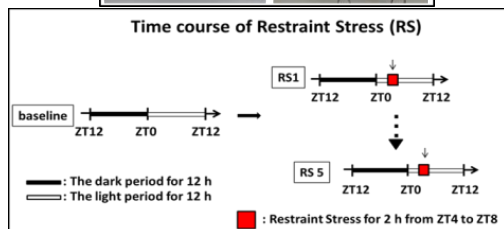
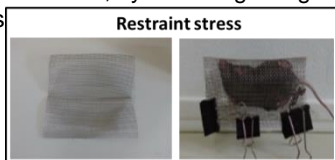
Upper row; voiding behavior in control mice. Voiding frequency (VF) in the light did not show any difference (middle panel). The bladder capacity in mice has the circadian rhythm, which was higher in the light than in the dark (right panel).

Lower row; voiding behavior in RS mice. Voiding frequency (VF) in the light increased significantly higher compared to the baseline. The circadian change of the bladder capacity disappeared after RS.

To reveal whether RS affects the alternation of gene expression rhythm and cause nocturia in mice, we investigated the expression rhythm of clock genes and mechano-sensors in the mice bladder mucosa after RS.

Methods

- Male C57BL/6 (WT) mice and Period2 luciferase knock-in mice (Per2::Luc) were used. Per2 is one of the clock genes and act as a negative transcriptional factor for clock-controlled-genes.
- Mice were bred under 12 h light/dark conditions.
- Mice were exposed to 2 h RS from ZT4 to ZT6, the sleep phase in mice, by enfolding using metal-mesh for 5 times

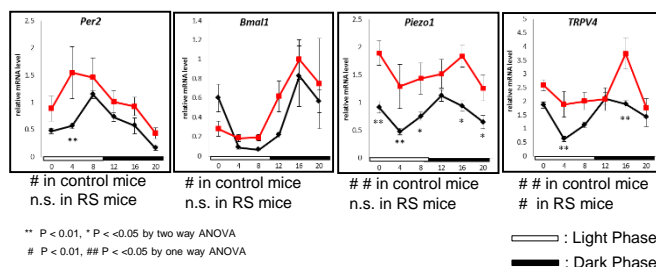


- Mice bladder was excised every 4 h from ZT0 in both control- and RS mice. mRNA in the mucosa of excised bladder was extracted, then the gene expression rhythms in clock genes and mechanosensor were measured using RT-PCR.
- Per2 expression in the bladder for Per2::Luc mice was measured using in vivo imaging.

Results

Experiment 1; RT-PCR

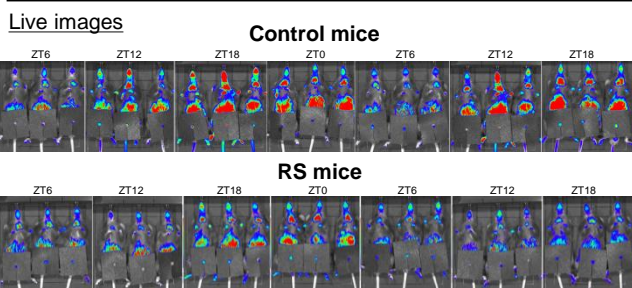
The mRNA expression rhythm in clock genes and mechano-sensors



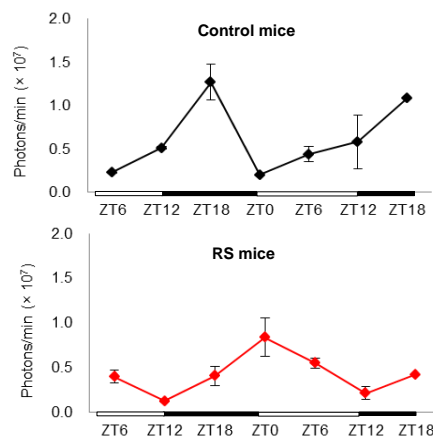
WT mice showed the time-dependent gene expression in clock genes and mechano-sensors. However, these rhythms were disrupted or the oscillation pattern was altered in RS mice.

Experiment 2; in vivo imaging

The Per2 expression rhythm in the mouse bladder



Total ROI Quantitation of Per2::Luc Bioluminescence in the bladder



Data were processed using an one-way ANOVA with Bonferroni's test. Data are presented as means ± SE.

WT mice also showed the robust circadian Per2 expression in the bladder. However, this rhythm was altered in RS mice (the peak was shifted), although circadian Per2 expression was maintained in RS mice. Moreover, the amplitude of bioluminescence in RS mice was lower than WT mice.

Conclusions

The circadian gene expressions in clock genes were altered by intermittent RS during the rest phase, sleep phase in mice, which might induce nocturia through the abnormal circadian expression of mechano-sensors.

These results indicated that exposure to stress could be one of the causes of nocturia. Further studies may provide new therapeutic strategies for nocturia.

References

- Ihara, T., Mitsui, T., Nakamura, Y. et al.: The oscillation of intracellular Ca²⁺ influx associated with the circadian expression of Piezo1 and TRPV4 in the bladder urothelium. Sci Rep, 2017.
- Tahara, Y., Shiraishi, T., Kikuchi, Y. et al.: Entrainment of the mouse circadian clock by sub-acute physical and psychological stress. Sci Rep, 2015