

Abstract

This study was conducted to examine if the circadian rhythm affects reflex micturition during cystometry (CMG) and voluntary voiding behavior in metabolic cage (MC). In MC analysis, urine volume per void (VV) was evaluated in 12:12-hour dark and light cycle. In both sexes, no differences in VV were found between the two periods. During CMG, male mice presented larger estimated voiding volume per micturition (eVV) in dark period than in light period ($P < 0.001$). In female mice, however, no difference in eVV was found between the two periods. Comparisons between eVV and VV in male showed great differences in both dark period and light period ($P < 0.001$ for each). Such comparisons in female showed only small difference in light period ($P < 0.05$) and not in dark period. The results suggest that the circadian rhythm affects reflex micturition cycle in male mice. Male mice have more robust voluntary regulatory system which is predominant over reflex mechanism.

Introduction

A recent study suggested that the circadian rhythm affects lower urinary tract function in mice, altering amount of genes expressed in the urothelium that are associated with afferent signal transduction [1]. However, a study using metabolic cage to examine voluntary voiding behavior in male mice showed that there is no difference in urine volume per micturition between dark period and light period of 12:12-hour cycle [2]. Meanwhile, such comparison based on the time cycle has not been conducted in cystometry evaluation. Thus, the aim of this study was to examine if the circadian cycle affects reflex micturition during cystometry in decerebrate, unanesthetized mice and to compare it with voluntary voiding behavior that was examined using metabolic cage.

Methods and Materials

We used 12-13 week-old C57BL/6N mice of both male and female ($n=48$ for each sex). In this study, a dual analysis of voiding behavior and reflex micturition was conducted to examine lower urinary tract function in these mice [2]. *For evaluating voluntary voiding behavior*, conscious mice were individually placed in metabolic cages (Fig. 1) for 5 days, and data of frequency-volume charts were collected in the last 2 days after 3 day-acclimation. *For assessing reflex micturition*, mice were decerebrated under sevoflurane anesthesia and cystometry recordings were conducted under unanesthetized conditions by continuously infusing saline (30 $\mu\text{l}/\text{min}$). *Evaluated parameters are:* urine volume per void (VV, μl) for metabolic cage study; and estimated voiding volume per micturition (eVV, μl) for cystometry study. eVV was calculated by 'actual collecting time' (s) x infusion rate ($\mu\text{l}/\text{min}$)/60' [3]. All values are expressed as mean \pm S.E.M. Statistical analyses were made using unpaired t-test, Mann-Whitney test, or Wilcoxon matched-pairs signed rank test, and $P < 0.05$ was considered significant. n.s., not significant. *which is calculated as ('2nd phase duration of previous bladder contraction' + 'intercontraction interval').

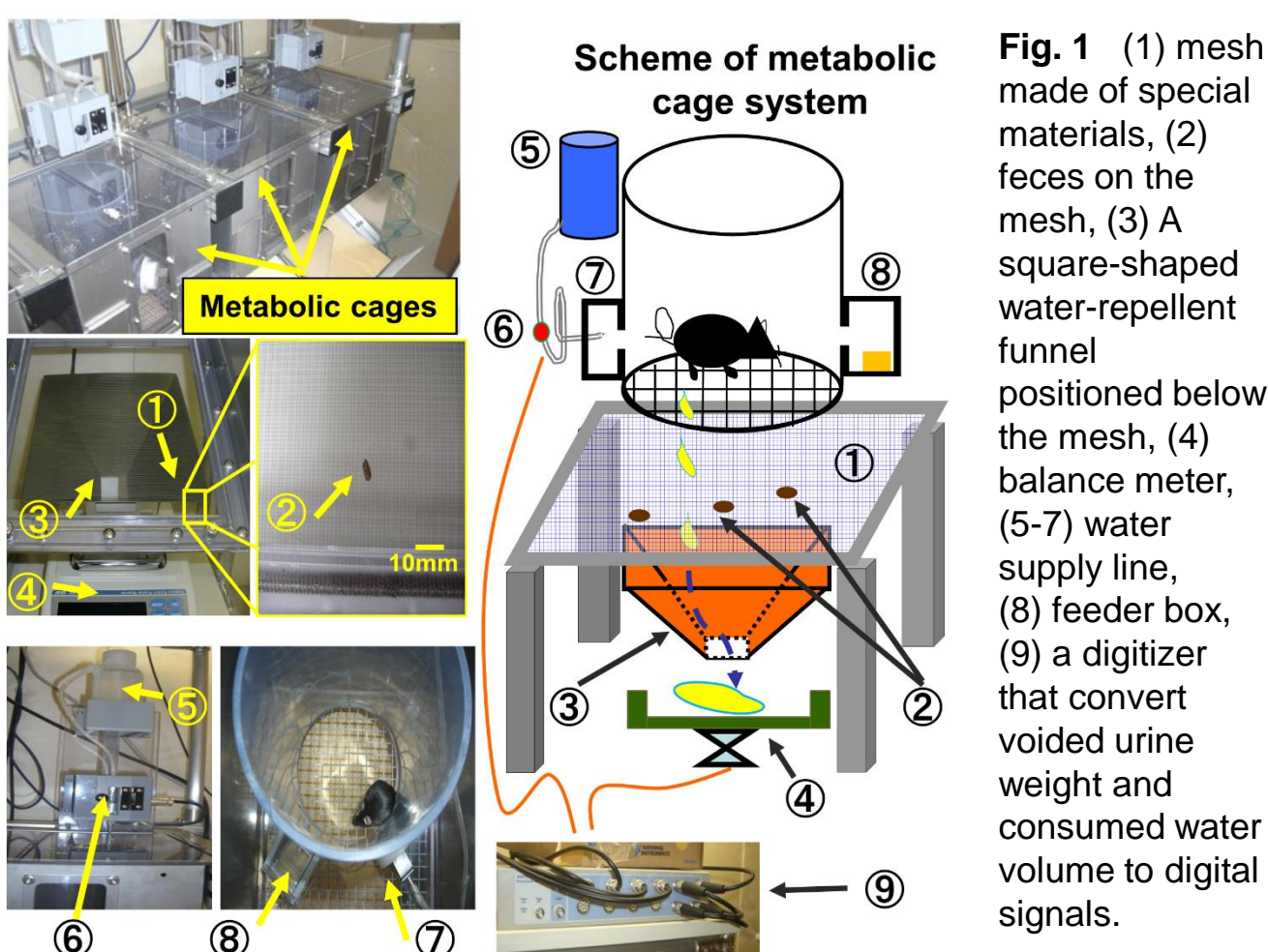


Fig. 1 (1) mesh made of special materials, (2) feces on the mesh, (3) A square-shaped water-repellent funnel positioned below the mesh, (4) balance meter, (5-7) water supply line, (8) feeder box, (9) a digitizer that convert voided urine weight and consumed water volume to digital signals.

Results

Graphic results of voluntary voiding behavior in metabolic cage (MC) and reflex micturition during cystometry (CMG) are indicated in Fig. 2.

Analysis of voluntary voiding behavior in metabolic cage (MC):

Urine volume per void (VV) was evaluated per 12 h in dark period and per 12 h in light period. In both sexes, no differences in VV were found between dark period and light period (male, $P > 0.9999$ and female, $P = 0.1563$, Wilcoxon matched-pairs signed rank test).

Evaluation of reflex activity of the lower urinary tract during cystometry (CMG):

Male mice presented larger estimated voiding volume per micturition (eVV) in dark period than in light period ($P = 0.0004$, unpaired t-test). In female mice, however, no difference in eVV was found between the two periods ($P = 0.1337$).

Comparisons between eVV during CMG and VV in MC in male showed great differences in both dark period and light period ($P < 0.001$ for each, Mann-Whitney test). Meanwhile, such comparisons in female showed small but significant difference in light period ($P = 0.0158$) and no difference in dark period ($P = 0.7882$).

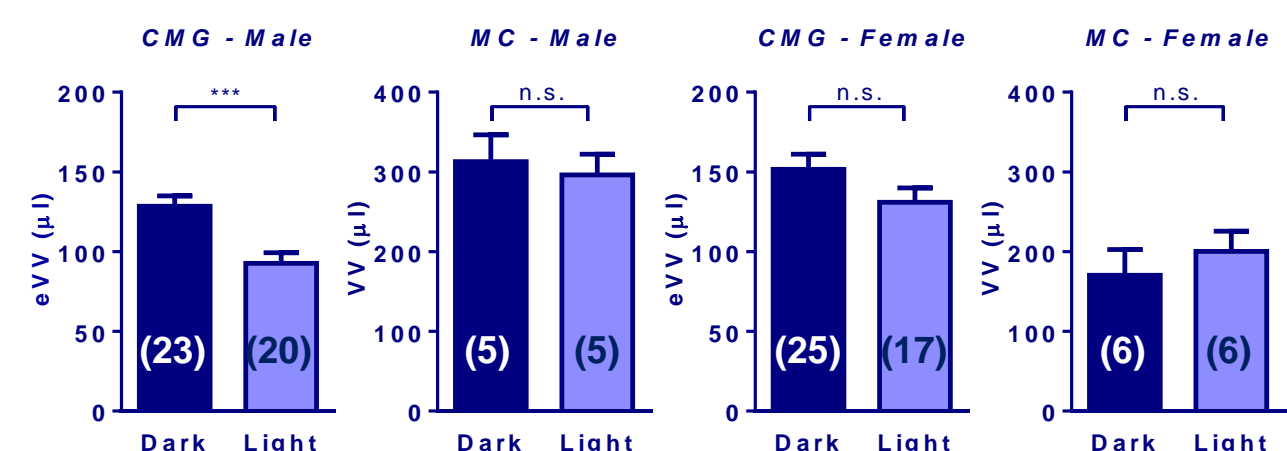


Fig. 2 Comparisons between the dark period and the light period in reflex micturition (i.e., during CMG) or voluntary voiding (i.e., behavior in MC). Number in parenthesis in each bar graph indicates number of animals used for analysis.

Discussion

In male mice, the 12:12-hour cycle affected functional bladder capacity (i.e., eVV) in reflex micturition during cystometry, whereas it had no influence on micturition volume at voluntary voiding. In female mice, the 12:12-hour cycle had no effects on either eVV during cystometry or VV at voluntary voiding. The results suggest that the circadian rhythm has an influence on lower urinary tract activity in male mice, affecting reflex micturition (i.e., under decerebrate, unanesthetized conditions), which would be dominated by consciousness (i.e., in voluntary behavior accompanying participation of the forebrain). However, it produced no effects on lower urinary tract activity in female via either reflex pathways or consciousness. Great differences between eVV (i.e., during cystometry) and VV (i.e., in metabolic cage) were found in male, whereas such differences were only a little or not found in female. It suggests that an inhibitory control of the forebrain more predominantly rules appropriate timing of urine release in male mice.

Conclusions

When performing cystometry in male mice, it is important to consider effect of the circadian rhythm on lower urinary tract function. Male mice execute more robust voluntary (i.e., forebrain) participation, which is predominant over reflex mechanism in control of lower urinary tract activity.

References

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