URINARY INCONTINENCE

Connexin43—clocking bladder rhythms in mice

New research has revealed a role for connexin43—a gap junction protein—in regulating functional bladder capacity. Additionally, the levels of connexin43 display circadian oscillation in wild-type mice, which is lost in *CRY*-null mice with dysfunctional biological clocks. These observations might have implications for understanding nocturnal enuresis in children and nocturia in the elderly.

Researchers from two realms—urology and chronobiology—came together to explore the overlap of the two specialties. Hitoshi Okamura's primary research focus has been on identifying clock-controlled genes and their roles in human diseases. Akihiro Kanematsu has primarily focused on functional bladder disorders and

the genes and proteins that underpin them. Their latest report in *Nature Communications* builds on their previous work that first identified clock gene activity in the mouse bladder.

As murine micturition volumes can be minute (sometimes as low as <50 µl), measuring the urine output of free-moving animals is no small challenge. To overcome this difficulty, the researchers developed the automated voided stain on paper (aVSOP) system that works by rolling pretreated absorbent paper (designed to colour once in contact with urine) underneath the mouse enclosure. aVSOP enabled the researchers to measure the temporal changes in urine volume, which was needed to study the urinary patterns of wild-type and genetically engineered mice.

Mice with decreased connexin43 levels (that is, partial knock-out *CX43^{+/-}* animals) voided more urine per micturition than their wild-type *CX43^{+/+}* counterparts. This finding suggests that connexin43 is involved in determining the functional capacity of the bladder. Additionally, urine volume per micturition in wild-type mice showed circadian oscillations that mirror those of healthy humans. The oscillations were not affected by light patterns (12 h light/12 h dark or constant dark), indicating that an internal biological clock (the work of various clock genes) regulates micturition rhythmicity by eliciting oscillations in connexin43 levels. Indeed, expression of

CX43 mRNA in the bladder showed very strong circadian oscillation that correlated with the changes in urine volume. This rhythm was abolished in mice null for *CRY1* and *CRY2*, two cryptochrome genes that regulate the circadian clock in mammals. Other clock genes, including *PER1* and *CLOCK*, also showed circadian expression profiles in the bladder, which further implicates chronobiological mechanisms with regulating normal bladder function.

These findings might help the detailed study of disorders such as nocturia and nocturnal enuresis. “Investigating the genes involved in enuresis might provide clues for treating the large number of patients who continue to have functional bladder disorders later in life,” Kanematsu explained. Indeed, as these disorders are characterized by a disconnection between cortical arousal in the brain, urine storage in the bladder and urine production rate by the kidneys, other organs must also be studied. “Our laboratory is also searching for clock-controlled genes involved in circadian urine production in the kidney,” added Okamura. The natural extension of this work is to develop treatments that target these mechanisms in each organ.

Mina Razzak

Original article Negoro, H. et al. Involvement of urinary bladder connexin43 and the circadian clock in coordination of diurnal micturition rhythm. *Nat. Commun.* doi:10.1038/ncomms1812

Automated voided stain on paper instrument. Courtesy of Akihiro Kanematsu.