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Shimizu N[1], Goins W[2], Takai S[3], Shimizu T[3], Wada N[3], Hirayama A[4], Uemura H[5], Tyagi P[3], Glorioso J[2], Yoshimura N[3]

1. *Dept of Urology- University of Pittsburgh, Kindai University Hospital, Faculty of Medicine,* ***2.*** *Dept of Microbiology & Molecular Genetics-University of Pittsburgh,* ***3.*** *Dept of Urology- University of Pittsburgh,* ***4.*** *Dept of Urology- Kindai University Nara Hospital, Faculty of Medicine,* ***5.*** *Dept of Urology-Kindai University Hospital, Faculty of Medicine*

HERPES SIMPLEX VIRUS (HSV) VECTOR-MEDIATED TRACING AND LABELLING OF DIFFERENT POPULATIONS OF BLADDER AFFERENT NEURONS IN NORMAL AND SPINAL CORD INJURED MICE

Hypothesis / aims of study

Chronic spinal cord injury (SCI) above the lumbosacral cord level induces neurogenic detrusor overactivity (NDO) in part due to enhanced excitability of bladder afferent pathways, especially in the C-fiber afferent population. In the previous research of neurogenic bladder dysfunction, rat models have often been used; however, the mouse model has become highly useful because of its feasibility for genetic modification and abundant databases although functional and morphological changes in bladder afferent pathways after SCI have not been well clarified in mice. Therefore, the present study using SCI mice examined; (1) the contribution of capsaicin-sensitive C-fiber afferent pathways to NDO as shown by nonvoiding contractions (NVCs) during cystometry, and (2) morphological changes in different populations of bladder afferent pathways after SCI by a new tracing and labelling method using replication-deficient herpes simplex virus (HSV) vectors encoding mCherry from one non-specific promoter (cytomegalovirus [CMV]) and 2 different neuronal cell-type-specific promotors (calcitonin gene-related peptide [CGRP], and neurofilament-200 [NF200]), which can express the mCherry fluorescent protein in non-selective, CGRP-positive C-fiber and NF200-positive A-fiber afferent neurons, respectively. (Fig.1)

Fig. 1: Neuronal Subtype Promoter HSV Vectors.

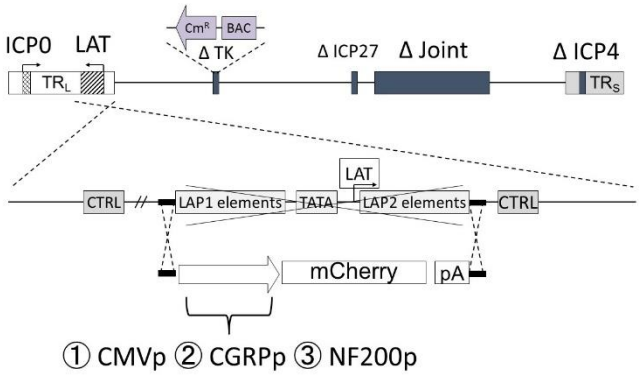
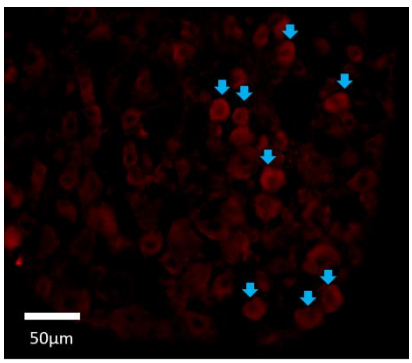


Fig. 2: CMVp vector-labelled neurons



Study design, materials and methods

Female C57BL/6N (8weeks) mice weighing 18-20g were used, and SCI mice underwent complete transection of Th8/9 spinal cord.

1. Cystometry: At 4 weeks after SCI, cystometory was performed under an awake condition in SCI mice with or without capsaicin pretreatment (50mg/kg) that was performed 4 days earlier.

2. HSV tracing study: Mice were divided into 6 groups; (1) spinal intact (SI) with CMVp-mCherry (n=3), (2) SI with CGRPp-mCherry (n=3), (3) SI with NF200p-mCherry (n=3), (4) SCI with CMVp-mCherry (n=3), (5) SCI with CGRPp-mCherry and (6) SCI with NF200p-mCherry (n=3) groups. At 2 weeks after SCI, under a laparotomy, a total of 20μl viral suspension containing 4×10^7 plaque-forming units [PFU]: CMVp-mCherry, 3×10^7PFU: CGRPp-mCherry or 2.36×10^7 PFU: NF200p-mCherry were injected into the bladder wall. In SI groups, mice underwent sham operation, and 2 weeks later the vectors were injected into the bladder wall as well. Then, 2 weeks after vector inoculation, SI and SCI mice were perfused with 4% paraformaldehyde solution, and L1 and L6 were removed bilaterally for immunofluorescent staining using anti-mCherry antibodies.

Results

1. The number of NVCs were significantly ($p<0.05$) less in capsaicin-pretreated SCI mice vs. untreated SCI rats.
2. The number of CMVp vector-labelled bladder afferent neurons (indicated by arrows in Fig. 2) were 6.6±0.4 (n=83 sections from 5 DRGs) and 5.7±0.4 cells (n=107 from 6 DRGs) per 10μm-thick L1 DRG section, and 16.4±1.0 (n=79 sections from 6 DRGs) and 14.0±0.8 cells (n=84 from 6 DRGs) per L6 DRG section in SI and SCI mice, respectively. There were no statistically significant differences between SI and SCI mice.
3. The numbers of CGRPp vector-labelled bladder afferent neurons were 5.0±0.3 (n=101 sections from 6 DRGs) and 8.8.±0.6 cells (n=94 from 6 DRGs) per L1 DRG section, and 6.8±0.6 (n=83 from 6 DRG) and 9.5±0.5 cells (n=94 from 6 DRGs) per L6 DRG section in SI and SCI mice, respectively. The number of labelled cells in L1 and L6 DRGs from SCI mice were significantly increased compared to the SI mice ($p<0.001$).
4. The numbers of NF200p vector-labelled bladder afferent neurons were 5.1±0.4 (n=82 sections from 6 DRGs) and 4.5.±0.3 cells (n=75 from 6 DRGs) per L1 DRG section, and 6.3±0.3 (n=89 from 6 DRGs) and 4.6±0.3 cells (n=94 from 6 DRGs) per L6 DRG section in SI and SCI mice, respectively. The number of labelled cells in L6 DRGs were significantly decreased compared to SI mice ($p<0.001$)
5. The histogram of size distributions of CGRPp vector-labelled C-fiber and NF200p vector-labelled A-fiber bladder afferent neurons shows an increase in the number of C-fiber neurons and a decrease in the number of A-fiber neurons in L6 DRGs after

SCI (Fig. 3). The median cell size of CGRPp vector-labelled bladder afferent neurons in L6 DRGs was shifted to a larger size ($p<0.05$) from 247.0 (SI) to 271.3 μm^2 (SCI) (Fig. 4).

Fig. 3: Cell size distribution of CGRPp and NF200p vector-labelled neurons (L6 DRG)

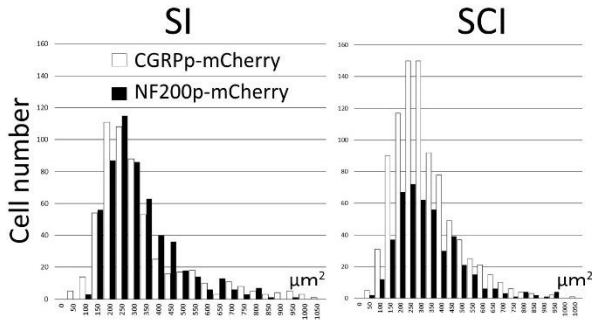
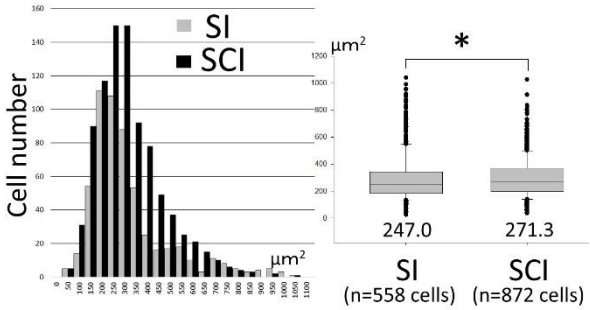


Fig. 4: Cell size distribution of CGRPp vector-labelled neurons (L6 DRG)



Interpretation of results

SCI-induced NDO was decreased by capsaicin-sensitive C-fiber desensitization, as evidenced by a significant reduction in NVCs in capsaicin-pretreated SCI mice. By using the HSV vector-mediated tracing technique, it was possible to distinguish C-fiber and A-fiber populations of bladder afferent neurons. The size of C-fiber and A-fiber bladder afferent neurons was smaller than rats, and there is a considerable overlap between C-fiber and A-fiber cell distribution in SI mice when compared to a previous rat study [2]. An increase in the number of CGRPp vector-labelled C-fiber cells with larger somal sizes and a decrease in the number of NF200p vector-labelled A-fiber cells without changing the total number of CMVp vector-labelled bladder afferent neurons in SCI mice indicate that SCI induces morphological plasticity in bladder afferent pathways such as expansion of C-fiber cell population with cell hypertrophy, which could underlie C-fiber hyperexcitability inducing NDO in SCI.

Concluding message

To our knowledge, this is the first study investigating morphological changes in different populations of bladder afferent neurons in mice using mCherry-encoding HSV vectors with cell type-specific promoters. We found that SCI can induce functional and morphological changes in bladder afferent pathways, especially in the C-fiber cell population, after SCI in mice. These findings help to understand the mechanism of neurogenic lower urinary tract dysfunction associated with SCI.

References

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Disclosures

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