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ORIGINAL ARTICLE

Erectile Dysfunction

Comparative study of intracavernous pressure and cavernous pathology after bilateral cavernous nerve crushing and resection in rats

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This study aimed to compare the effects of bilateral cavernous nerve crushing (BCNC) and bilateral cavernous nerve resection (BCNR) on intracavernous pressure (ICP) and cavernous pathology in rats and to explore the optimal treatment time for the BCNC and BCNR models. Seventy-two male rats aged 12 weeks were randomly divided into three equal groups: Sham (both cavernous nerves exposed only), BCNC (BCN crushed for 2 min), and BCNR (5 mm of BCN resected). Erectile function was then measured at 1 week, 3 weeks, and 5 weeks after nerve injury, and penile tissues were harvested for histological and molecular analyses by immunohistochemistry, immunofluorescence, Western blot, and cytokine array. We found that erectile function parameters including the maximum, area, and slope of ICP/mean arterial pressure (MAP) significantly decreased after BCNR and BCNC at 1 week and 3 weeks. At 5 weeks, no significant differences were observed in ICP/MAP between the BCNC and Sham groups, whereas the ICP/MAP of the BCNR group remained significantly lower than that of the Sham group. After BCNC and BCNR, the amount of neuronal-nitric oxide synthase-positive fibers, smooth muscle cells, and endothelial cells decreased, whereas the amount of collagen III content increased. These pathological changes recovered over time, especially in the BCNC group. Our findings demonstrate that BCNC leads to acute and reversible erectile dysfunction, thus treatment time should be restricted to the first 3 weeks post-BCNC. In contrast, the self-healing ability of the BCNR model is poor, making it more suitable for long-term treatment research.

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Keywords: cavernous nerve crushing; cavernous nerve resection; erectile dysfunction; intracavernous pressure

INTRODUCTION

Erectile dysfunction (ED) is a common complication after radical prostatectomy (RP),[1,2] which severely affects patient's quality of life. Post-radical prostatectomy erectile dysfunction (pRP-ED) is caused by cavernous nerve (CN) injury – the CN contains sympathetic and parasympathetic nerve fibers that transmit sex drive and release different neurotransmitters.[3] At present, pRP-ED is commonly attributed to the weakened relaxation ability of cavernous smooth muscle and loss of endothelial cells and an aggravation of fibrosis after CN injury.[4,5] However, the detailed mechanism of pRP-ED is poorly understood.[6]

A widely used animal model of pRP-ED is the rat model of CN injury because it can physiologically mimic human CN injury and is more economical compared with larger animal models.[7] Furthermore, the CNs in rats are distinct entities that clearly branch off the major pelvic ganglions (MPGs).[8] In rat models, there are many forms of nerve injury used to mimic the damage that can happen in pRP-ED, including crushing, resection, cautery, and freeze injury.[9,10] Among these, the most commonly used is the bilateral cavernous nerve crushing (BCNC) model; however, the severity of CN injury achieved with this model is inconsistent among reports. This inconsistency is mainly due to crushing time ranging from 15 s to 2 min and to the variety of instruments used in crushing, such as forceps, hemostats, and micro needle holders. Moreover, the end-point analysis time of such studies has ranged from 1 day to 8 weeks.[11] Therefore, the reported percentage reduction of intracavernous pressure/mean arterial pressure (ICP/MAP) after CN injury ranges from 34% to 84%, and the percentage improvement after therapeutic intervention ranges from 5% to 250%.[12–14] This variable use of the BCNC model creates false positives and leads to incorrect conclusions about the efficacy of ED treatment. To address this issue, we set out to investigate the changes in ICP/MAP and the pathology of the corpus cavernosum in rats at three predetermined time points after BCNC and bilateral cavernous nerve resection (BCNR). We aim to provide insight into the future intervention of ED.

MATERIALS AND METHODS

Experimental design

Seventy-two Sprague–Dawley male rats aged 12 weeks were randomly divided into three groups (n = 24): Sham (both CNs exposed only),

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BCNC (bilateral CN crush), and BCNR (5 mm of the cavernous nerves were resected bilaterally). Eight rats were taken from each group to measure erectile function at 1 week, 3 weeks, and 5 weeks after nerve injury. Penile tissues were harvested for histological and molecular analyses by immunohistochemistry, immunofluorescence, Western blot, and cytokine array.

Bilateral cavernous nerve injury

Rats were anesthetized by intraperitoneal injection of 5% pentobarbital sodium (45 mg kg^{-1}; Sigma-Aldrich, St. Louis, MO, USA). The bladder and prostate were exposed via a midline abdominal incision, and the MPGs and CNs were identified posterolateral to the prostate. The CN injury point was 5 mm distal to the MPG. In the BCNC group, CNs were crushed by applying hemostatic forceps (RS-7116, Roboz, Gaithersburg, Germany) for 2 min.[14] In the BCNR group, bilateral cavernous nerve injury (BCNI) was created by resecting 2–3 mm of the CNs.[15]

Assessment of erectile function

Assessment of erectile function was performed at 1 week, 3 weeks, and 5 weeks after CN injury. ICP and MAP were measured as previously described.[16] Briefly, the rats were first anesthetized by intraperitoneal injection of 5% pentobarbital sodium (45 mg kg^{-1}). The right carotid artery and the penis were then exposed and separated, and two 24G needles connected to PE-50 tubes (Becton, Dickinson and Company, Franklin lakes, NJ, USA) with heparinized saline (250 IU ml^{-1}; Shuanghe Corporation, Beijing, China) were inserted into the corpus cavernosum and left carotid artery. The other end of each PE-50 tube was connected to a pressure acquisition system (MP150; BIOPAC system Inc., Goleta, CA, USA). The CN was then exposed as described previously, and electrostimulation (5 V, 20 Hz, pulse width 1 ms, duration 60 s) was applied with a bipolar hook electrode (EL452, BIOPAC system Inc.). During electric field stimulation (EFS) of the CN, the ratio of ICP/MAP was calculated to normalize for variations in systemic blood pressure. The parameters used for comparison were ICP/MAP maximum, area under the ICP/MAP curve, and the slope for ICP/MAP to reach 80% of the maximal ICP/MAP when electrically stimulated.

Histology

Penile midshaft tissue was freshly harvested and divided into three parts transversally. One part was cryopreserved for protein extraction, one part was cryo-embedded for immunofluorescent (IHF) staining, and the final part was paraffin embedded for immunohistochemical (IHC) staining. For IHC and IHF, tissue sections used were 5 μm thick. Dilutions of primary antibodies (incubated overnight at 4°C) were as follows: alpha-smooth muscle actin (α-SMA; 1:3000; ab124964, Abcam, Cambridge, UK); neuronal-nitric oxide synthase (n-NOS; 1:500; ab95436, Abcam); neurofilament medium (NF; 1:500; ab7794, Abcam); and rat endothelial cell antigen-1 (Reca-1; 1:500; HIS52, Bio-Rad, Hercules, CA, USA). Appropriate species-directed secondary antibodies were applied to the sections for 90 min at room temperature; for IHF: α-SMA (1:500; A11012, Invitrogen, Carlsbad, CA, USA), n-NOS (1:500; A11001, Invitrogen); NF (1:500; A11012, Invitrogen), and Reca-1 (1:500; A11001, Invitrogen); and for IHC: α-SMA (ZB-2301, ZSGB-BIO, Beijing, China), nNOS (ZB-2305, ZSGB-BIO), NF (ZB-2301, ZSGB-BIO), and Reca-1 (ZB-2305, ZSGB-BIO). Semi-quantitative analysis

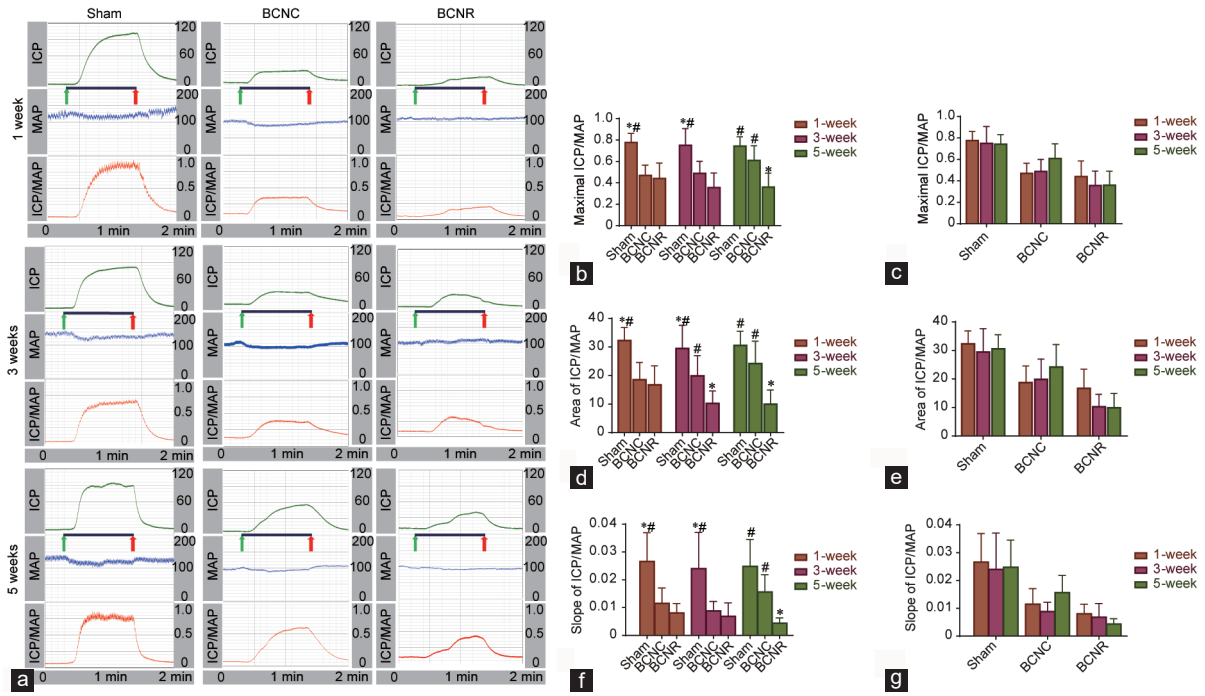

Figure 1: Assessment of erectile function by electric field stimulation at 1 week, 3 weeks, and 5 weeks after bilateral cavernous injury. (**a**) Assessment of ICP and MAP; ICP was normalized to MAP, and the ICP/MAP curve was calculated. (**b**) Comparison between treatment groups at each time point for the maximum of ICP/MAP. (**c**) Comparison within treatment groups at 1 week, 3 weeks, and 5 weeks for the maximum of ICP/MAP. (**d**) Comparison between treatment groups at each time point for the area of ICP/MAP. (**e**) Comparison within treatment groups at 1 week, 3 weeks, and 5 weeks for the area of ICP/MAP. (**f**) Comparison between treatment groups at each time point for the slope of ICP/MAP. (**g**) Comparison within treatment groups at 1 week, 3 weeks, and 5 weeks for the slope of ICP/MAP. $^{*}P < 0.05$ means BCNR and Sham versus the BCNC group; $^{\#}P < 0.05$ means BCNC and Sham versus the BCNR group. BCNR: bilateral cavernous nerve resection; BCNC: bilateral cavernous nerve crushing; ICP: intracavernous pressure; MAP: mean arterial pressure.

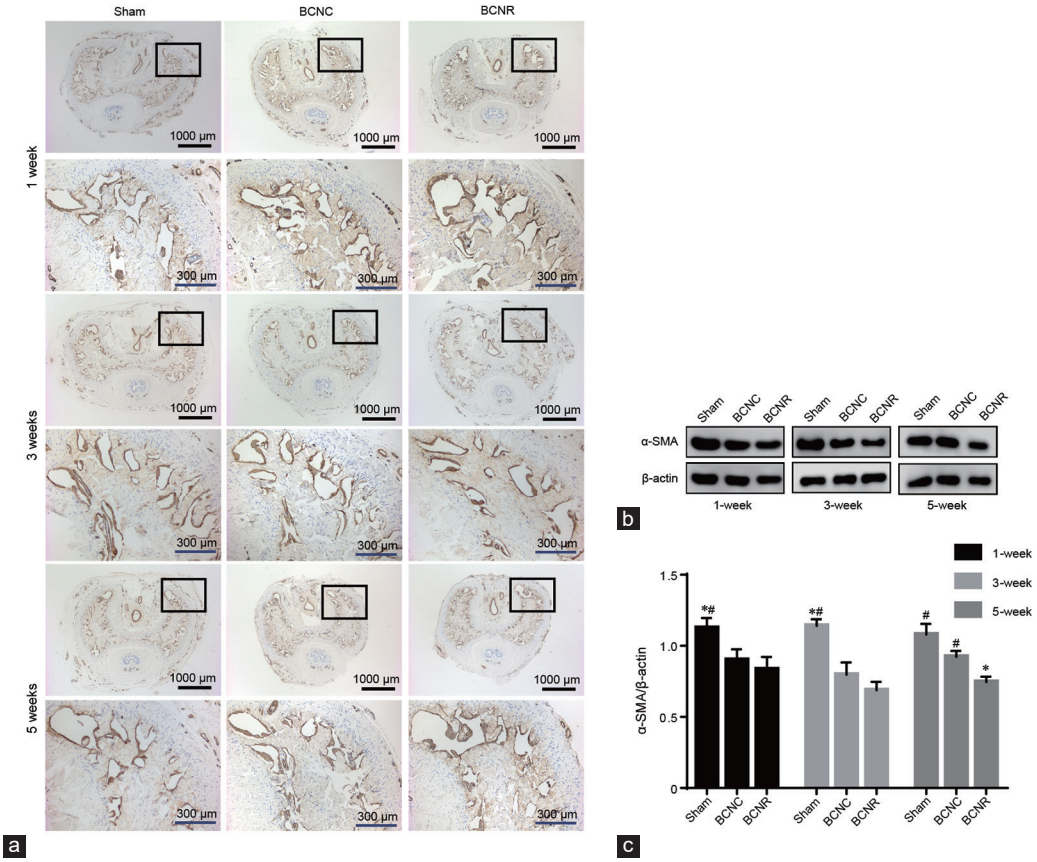

Figure 2: Changes in α-SMA content in the corpus cavernosum at 1 week, 3 weeks, and 5 weeks after bilateral cavernous injury. (**a**) Representative immunohistochemistry images of α-SMA in 5-μm cross sections of paraffin-embedded penile midshaft. (**b**) Western blot analysis of α-SMA expression. (**c**) The ratio of α-SMA to β-actin detection by Western blot. $^{*}P < 0.05$ means BCNR and Sham versus the BCNC group; $^{\#}P < 0.05$ means BCNC and Sham versus the BCNR group. BCNR: bilateral cavernous nerve resection; BCNC: bilateral cavernous nerve crushing; α-SMA: alpha-smooth muscle actin.

was performed to evaluate the intensity of α-SMA, nNOS, NF, and Reca-1 staining using Image Pro Plus software (version 6.0, Media Cybernetics Corporation, Houston, TX, USA).

Western blot

Penis tissue was mixed with RIPA Lysis Buffer (CW2333, CWBIO, Beijing, China) containing protein inhibitor cocktail (Sigma-Aldrich). This homogenate was then centrifuged at 20 000 *g* (ST16R, Thermo Scientific, Waltham, MA, USA) for 10 min at 4°C, and the supernatants were analyzed using the BCA protein assay. Proteins were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to a polyvinylidene fluoride membrane (Millipore Corp., Bedford, MA, USA). After incubation in a blocking solution of 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h at room temperature, the membranes were incubated with primary antibodies for α-SMA (1:20 000; ab124964, Abcam) and GAPDH (1:10 000; CWBIO). The membranes were then incubated with secondary antibody for 90 min at room temperature. Detection and image acquisition was achieved directly in a chemiluminescence-compatible digital imaging system (C-DiGit Blot Scanner, LI-COR Biosciences, Cambridge, UK).

Cytokine arrays

Penile tissue was sampled in the 3rd week after BCNI surgery, and cytokine antibody arrays were processed using the Proteome Profiler Rat XL Cytokine Array Kit (Cat. No. ARY030; R&D Systems, Minneapolis, MN, USA). After preparation, the concentration of penis proteins was adjusted to 500 μg ml^{-1}. Subsequent steps were carried out according to the manufacturer's instructions. Cytokines and chemokines in these blots were detected digitally by C-digit machine (LI-COR Biosciences, Cambridge, UK). Digital copies were quantified using Image J software (version 1.47, National Institutes of Health, Bethesda, MD, USA).

Statistical analyses

Data were expressed as means ± standard deviation. Differences between multiple groups were compared by one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparisons test using SPSS statistical software (version 13.0, SPSS, Chicago, IL, USA). The criterion for statistical significance was $P < 0.05$.

RESULTS

Assessment of erectile function

The maximum, slope, and area of ICP/MAP represent erectile rigidity, erectile speed, and total blood flow during erection, respectively. These parameters statistically significantly decreased in BCNR and BCNC at 1 week and 3 weeks after injury (all $P < 0.05$). However, at 5 weeks, the erectile function of the BCNC group was statistically significantly higher than that of the BCNR group ($P < 0.05$), and there was no statistically significant difference between the BCNC group and the control (Sham) group ($P > 0.05$; **Table 1** and **Figure 1**).

Changes in smooth muscle, endothelial cells, and collagen content in the corpus cavernosum

Smooth muscle content was assessed by IHC staining and Western blot for α-SMA. At 1 week and 3 weeks after BCNC or BCNR, the smooth muscle content of the penis decreased compared with that in the Sham group. However, at week 5, there was no significant difference in the expression of α-SMA in the BCNC group compared with that in the Sham group (P>0.05), and the expression of α-SMA in the BCNC group was significantly higher than that in the BCNR group (P<0.05) (**Figure 2**). Immunofluorescence staining of cavernous endothelial cells with an antibody to Reca-1 showed that the mean density of Reca-1 statistically significantly decreased in the BCNC and BCNR groups at 1 week, 3 weeks, and 5 weeks, compared with that in the Sham group (all P < 0.05). In addition, compared with BCNC, endothelial cell content in the BCNR group decreased statistically significantly (P < 0.05) and did not recover within 5 weeks after injury (**Figure 3**). Collagen III in the corpus cavernosum was also evaluated by IHC staining. Compared with the BCNC group, the expression of collagen III in the BCNR group increased statistically significantly (P < 0.05) and the deposition of collagen III became more severe over time (**Figure 4**).

Table 1: Maximum, area, and slope of intracavernous pressure/mean arterial pressure

Variate	*Sham*	*BCNC*	*BCNR*
1 week, mean±s.d.			
Max	0.777±0.022*,#	0.470±0.048	0.440±0.060
Area	32.27±1.18*,#	18.50±3.04	16.73±2.73
Slope	0.0267±0.003*,#	0.011±0.003	0.008±0.001
3 weeks, mean±s.d.			
Max	0.738±0.034*,#	0.488±0.057	0.356±0.060
Area	29.46±1.70*,#	19.85±3.55#	10.21±1.97*
Slope	0.0240±0.003*,#	0.009±0.002	0.007±0.002
5 weeks, mean±s.d.			
Max	0.742±0.022#	0.645±0.065#	0.360±0.065*
Area	30.56±1.24#	24.17±3.95#	9.92±2.52*
Slope	0.0248±0.002#	0.016±0.003#	0.004±0.001*

*P<0.05 means BCNR and Sham versus the BCNC group at 1 week, 3 weeks, and 5 weeks; #P<0.05 means BCNC and Sham versus the BCNR group at 1 week, 3 weeks, and 5 weeks. Max: the maximum of the ICP/MAP curve; area: the area under the ICP/MAP curve; slope: slope for the ICP/MAP to reach 80% of the maximal ICP/MAP during EFS; ICP: intracavernous pressure; MAP: mean arterial pressure; BCNR: bilateral cavernous nerve resection; BCNC: bilateral cavernous nerve crushing; EFS: electric field stimulation; s.d.: standard deviation

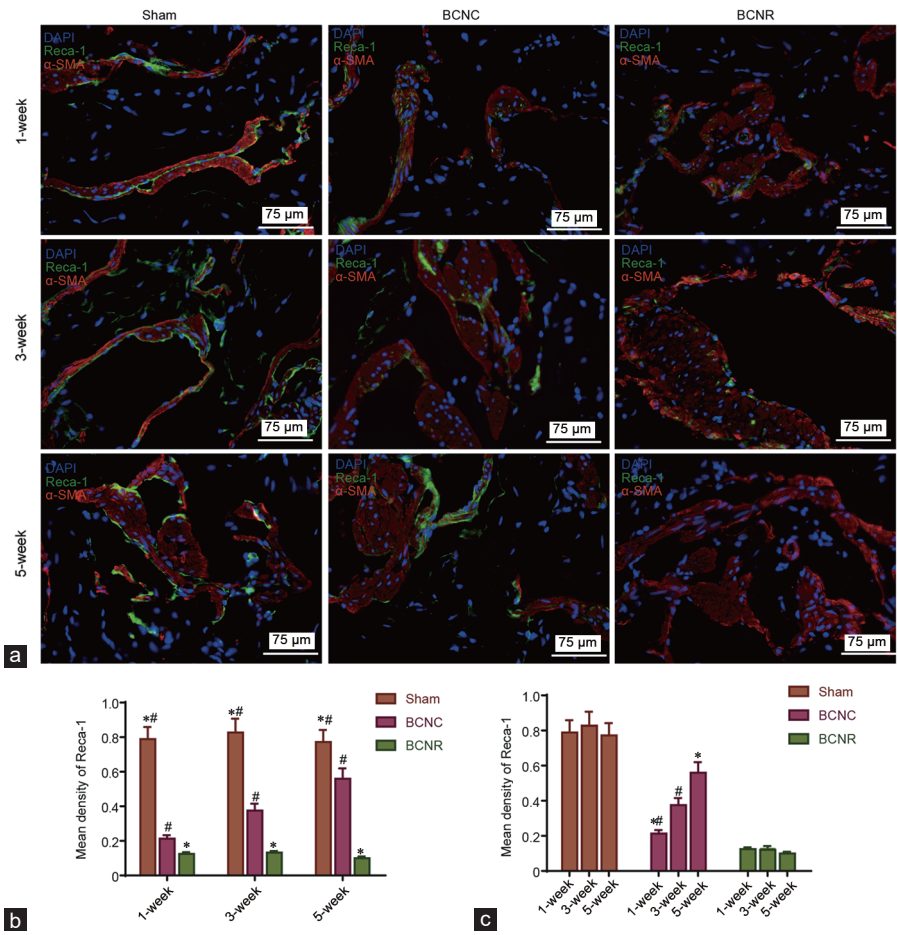

Figure 3: Changes in endothelial cells in the corpus cavernosum at 1 week, 3 weeks, and 5 weeks after bilateral cavernous injury. Rat endothelial cell antigen-1 (Reca-1) is a specific marker of endothelial cells. α-SMA is a specific marker of smooth muscle. (**a**) Representative immunofluorescence images of α-SMA (red) and Reca-1 (green) expression in 5-μm cross sections of cryo-embedded penile midshaft. (**b**) Comparison of semiquantitative mean densities of Reca-1 expression between treatment groups at each time point. *P < 0.05 means BCNR and Sham versus the BCNC group; #P < 0.05 means BCNC and Sham versus the BCNR group. (**c**) Comparison of semi-quantitative mean densities of Reca-1 expression within treatment groups at each time point. *P < 0.05 means week 1 and week 5 versus week 3; #P < 0.05 means week 1 and week 3 versus week 5. BCNR: bilateral cavernous nerve resection; BCNC: bilateral cavernous nerve crushing; α-SMA: alpha-smooth muscle actin; DAPI: 4',6-diamidino-2-phenylindole.

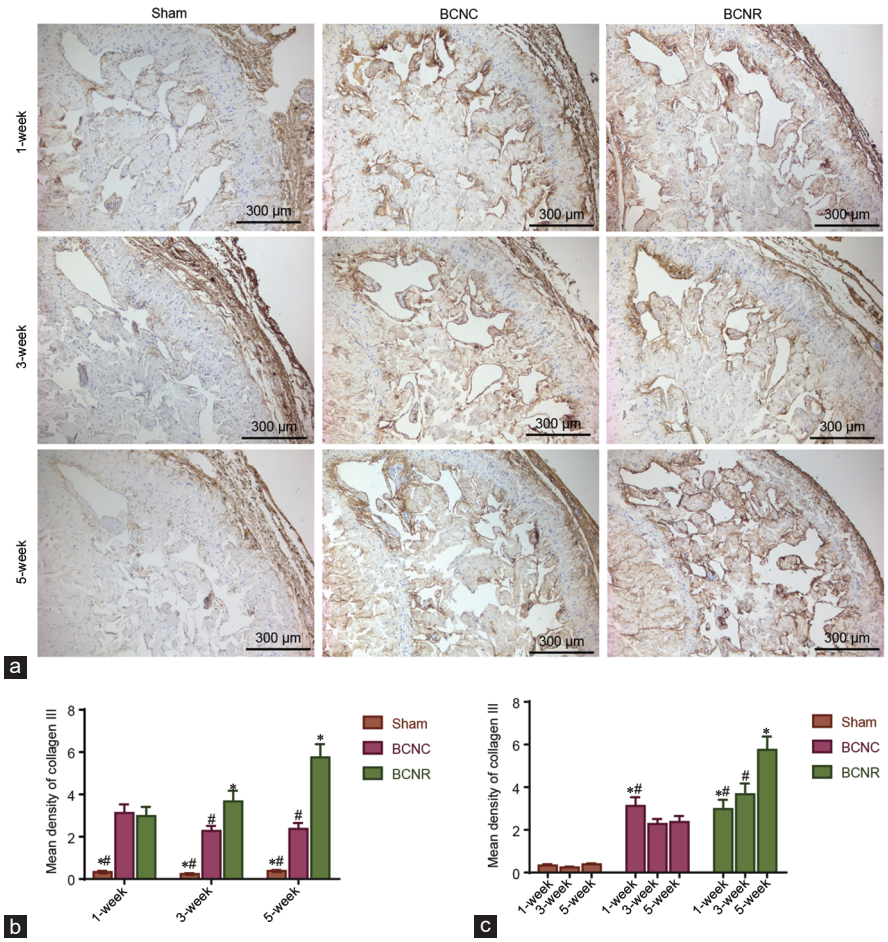

Figure 4: Changes in collagen III in the corpus cavernosum at 1 week, 3 weeks, and 5 weeks after bilateral cavernous injury. (**a**) Representative immunohistochemistry images of collagen III in 5-μm cross sections of paraffin-embedded penile midshaft. (**b**) Comparison of semi-quantitative mean densities of collagen III expression between treatment groups at each time point. $^{*}P < 0.05$ means BCNR and Sham versus the BCNC group; $^{\#}P < 0.05$ means BCNC and Sham versus the BCNR group. (**c**) Comparison of semi-quantitative mean densities of collagen III expression within treatment groups at each time point. $^{*}P < 0.05$ means week 1 and week 5 versus week 3; $^{\#}P < 0.05$ means week 1 and week 3 versus week 5. Scale bars = 300 μm. BCNR: bilateral cavernous nerve resection; BCNC: bilateral cavernous nerve crushing.

Changes in nNOS- and NF-positive nerves

nNOS-positive nerve endings within the corpus cavernosum statistically significantly decreased after BCNI ($P < 0.05$). In the weeks following injury, the number of nNOS-positive nerves in the BCNC group increased and was statistically significantly higher than that in the BCNR group ($P < 0.05$; **Figure 5**). In the MPG and penile dorsal nerve cord, both BCNC and BCNR resulted in a statistically significant decrease in NF- and nNOS-positive nerves (all $P < 0.05$). Recovery of NF and nNOS expression was observed in both groups over time, with the nerve regeneration ability of the BCNC group being statistically significantly better than that of the BCNR group ($P < 0.05$; **Supplementary Figure 1** and **2**).

Cytokine arrays of penis proteins

Rat cytokine arrays showed that the expression of epidermal growth factor (EGF) was statistically significantly lower in the BCNC and BCNR groups than that in the Sham group (both $P < 0.05$). In contrast, the expression of C-C motif ligand 21 (CCL21) and fibroblast growth factor (FGF) statistically significantly increased in the BCNC and BCNR groups (all $P < 0.05$). Compared with BCNC, the expression of FGF and CCL21 in the BCNR group was statistically significantly higher (both $P < 0.05$) and the expression of EGF was statistically significantly lower ($P < 0.05$; **Supplementary Figure 3**).

DISCUSSION

The BCNR and BCNC rat models are the most commonly used animal models for the study of pRP-ED. BCNR is performed by resecting 5 mm of CN distal to the MPG, while the execution of BCNC has many variables including crush duration and crushing device.[11] The occurrence of ED after BCNC varies greatly because of this heterogeneity, which makes it challenging to study the mechanism of ED and determine treatment time when using this model. To evaluate and compare erectile function and pathological changes after CN injury in both BCNR and BCNC, we thus carried out a time course study using predetermined time points.

Specifically, we measured ICP and pathological changes at 1 week, 3 weeks, and 5 weeks after BCNC and BCNR. Our results revealed that both BCNC and BCNR are acute injuries and that BCNC mimics a mild, fully reversible nerve injury, whereas BCNR represents severe, permanent nerve damage (in the 5-week period we examined). Moreover, we found that erectile function could return to normal before full recovery of the pathological injury. Therefore, we recommend that the end-point analysis time for BCNC should

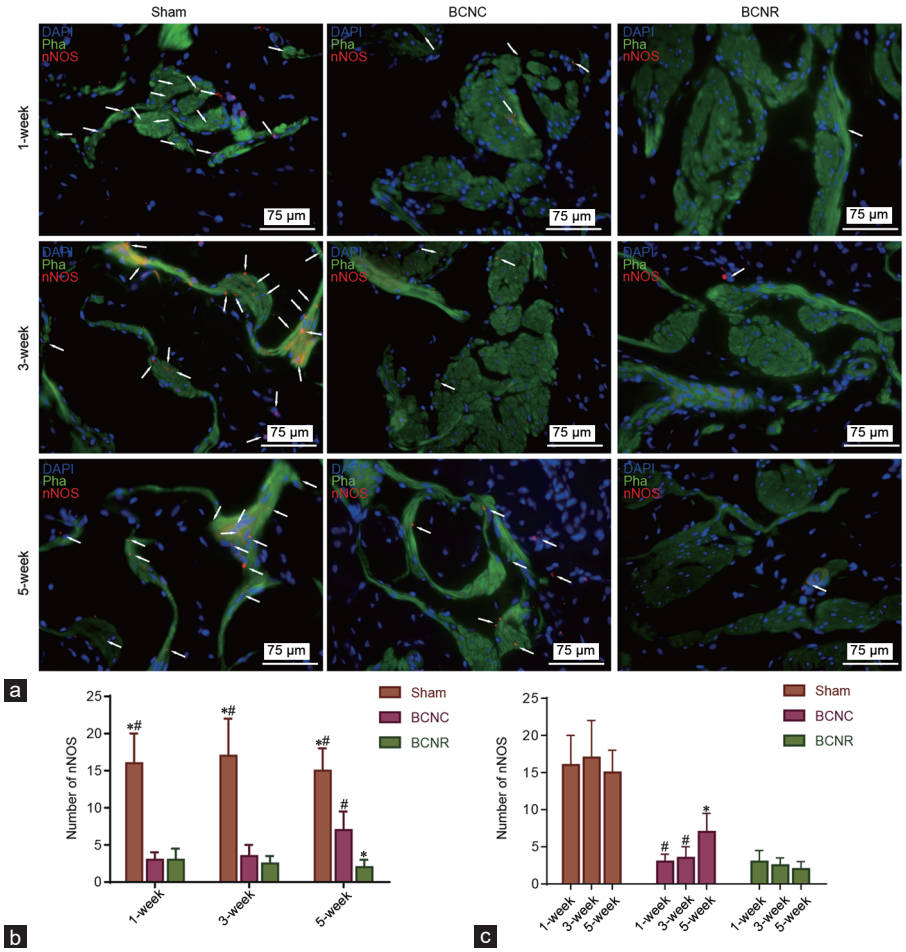

Figure 5: Changes in nNOS-positive nerves in the corpus cavernosum at 1 week, 3 weeks, and 5 weeks after bilateral cavernous injury. (**a**) Representative immunofluorescence images of nNOS (red) and phalloidin (Pha, green) in 5-μm cross sections of cryo-embedded penile midshaft at ×400. (**b**) Comparison of semi-quantitative analysis of nNOS expression between treatment groups at each time point. $^{*}P < 0.05$ means BCNR and Sham versus the BCNC group; $^{\#}P < 0.05$ means BCNC and Sham versus the BCNR group. (**c**) Comparison of nNOS expression within treatment groups at each time point. $^{*}P < 0.05$ means week 1 and week 5 versus week 3; $^{\#}P < 0.05$ means week 1 and week 3 versus week 5. BCNR: bilateral cavernous nerve resection; BCNC: bilateral cavernous nerve crushing; nNOS: neuronal-nitric oxide synthase; DAPI: 4',6-diamidino-2-phenylindole.

be strictly within 3 weeks of injury. However, for the BCNR model, recovery of erectile function and pathological damage was relatively slow, and thus this model can be used for studying the long-term intervention of ED.

Many investigators have noted that the relaxation of corpus cavernosum smooth muscle cells (CCSMCs) and endothelial cells is the most important component of erection physiology.[17–19] After CN injury, the number of nNOS-positive fibers decreases, resulting in a decreased relaxation ability of CCSMCs. Long-term ischemia and hypoxia of the penile cavernous body can also cause damage to CCSMCs and endothelial cells, including irreversible damage such as severe fibrosis.[4,5,15] In addition, Yang *et al.*[20] found that under hypoxic conditions, the phenotype of CCSMCs changes from contractile smooth muscle to synthetic smooth muscle, and their contractile and relaxation abilities are reduced. In our experiment, recovery of erectile function was consistent with pathological changes in the corpus cavernosum. For BCNC, erectile function and smooth muscle cells recovered to normal at 5 weeks, endothelial cells increased over time, and fibrosis gradually reduced. In comparison, for BCNR, erectile function and pathological damage remained severe at 5 weeks and showed no tendency to recover.

Compared with that of CCSMCs and endothelial cells, the regeneration speed of injured nerve fibers was relatively slow in the BCNC group. Notably, Lin *et al.*[21] observed that the regeneration rates of sympathetic and parasympathetic nerve fibers were different in injured CNs. These authors proposed that the rate of regeneration of parasympathetic nerves (causing relaxation of CCMSCs) was slower than that of sympathetic nerves, which leads to the total number of nNOS-positive nerve fibers in a reconstructed CN being less than that in normal CNs. This is consistent with our experimental results. In addition, erectile ability, including erectile speed, erectile rigidity, and total blood flow during erection, can return to normal before nerve reconstruction is completed. This means that there is a "threshold" presence of nNOS-positive CN fibers required to initiate the erection process, which explains the restoration of erectile capability before complete CN regeneration.

BCNC and BCNR simulate the preservation and nonpreservation of CN during radical prostatectomy, respectively. When the continuity of the CN is not preserved, the pathological changes of the corpus cavernosum are immediately severe and irreversible. Prudovsky *et al.*[22] proposed that FGF could be released under hypoxic conditions. FGF can stimulate the proliferation of fibroblasts that give rise to granulation tissue early in the

wound-healing process. In this study, cytokine arrays confirmed that the expression of FGF and CCL21 in the BCNR group was significantly higher than that in the BCNC group. Many studies have also found that long-term ischemia of the corpus cavernosum can lead to endothelial cell apoptosis.[23,24] This is consistent with the decrease in EGF that we observed in this study, as EGF has been suggested to play a key role in preventing apoptosis by increasing the expression of anti-apoptotic protein B-cell lymphoma-extra large (Bcl-XL) and preventing mitochondrial dysfunction induced by apoptotic members of the Bcl-2 family.[25]

Finally, we note several limitations of our study: we did not determine erectile function and pathological changes after BCNC and BCNR for longer than 5 weeks, and as the valid ED period of BCNC that we have determined is relevant to experimental conditions, it may change if different parameters of BCNC are used.

CONCLUSIONS

BCNC-induced ED in rats is reversible. The valid period of the BCNC model is within 3 weeks after BCNC, to avoid the spontaneous recovery of pathological changes. In contrast, recovery of erectile function and pathological damage in the BCNR model is relatively slow, thus this model could be used for studying long-term interventions for ED.

AUTHOR CONTRIBUTIONS

RLG and DF designed the study; ML, YMY, and BCY performed the experiments; ZCX, SJG, and HXL analyzed the data; and ML and YMY wrote the paper. All authors have read and approved the final manuscript, and agreed with the order of presentation of the authors.

COMPETING INTERESTS

All authors declare no competing interests.

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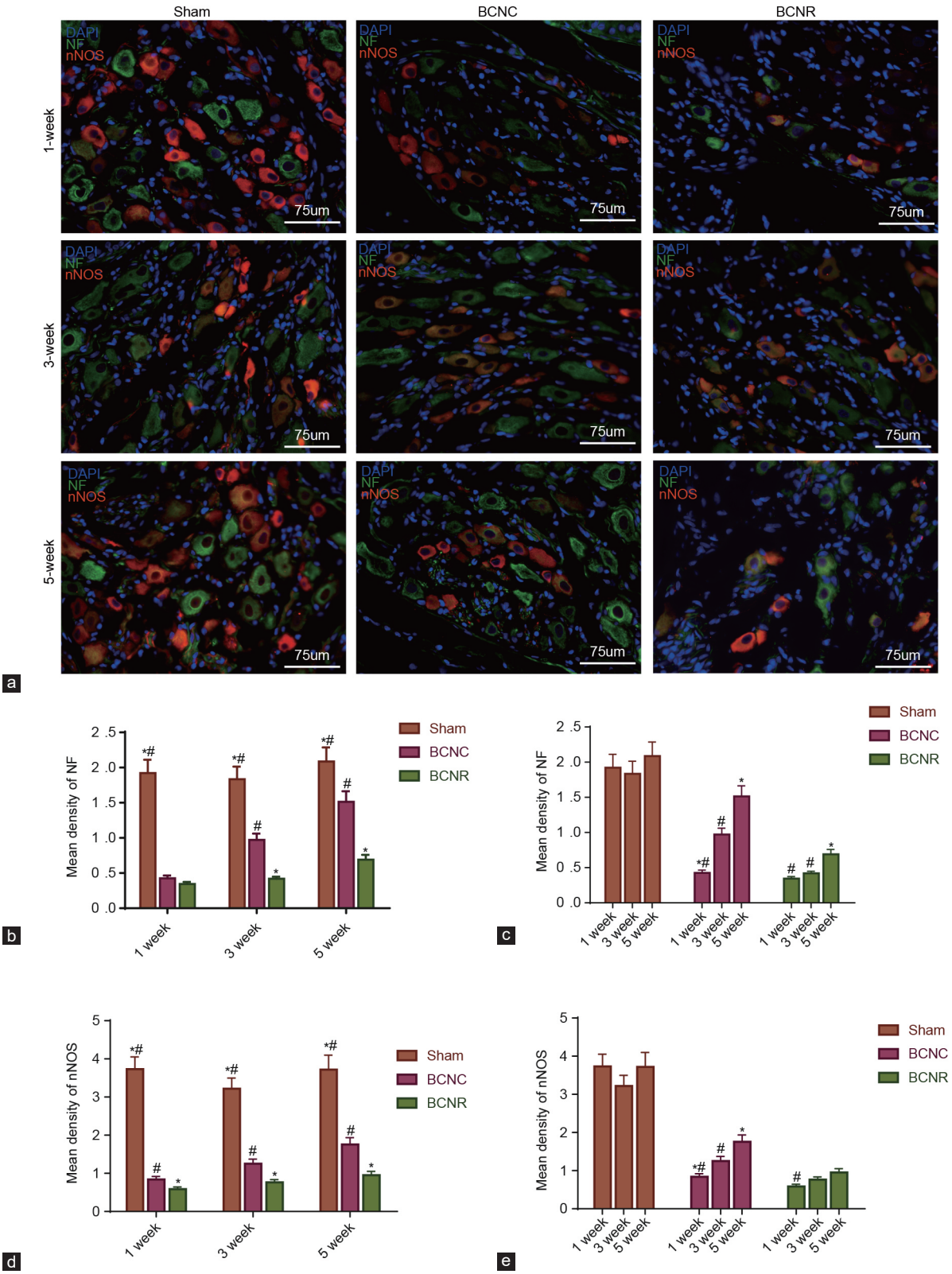
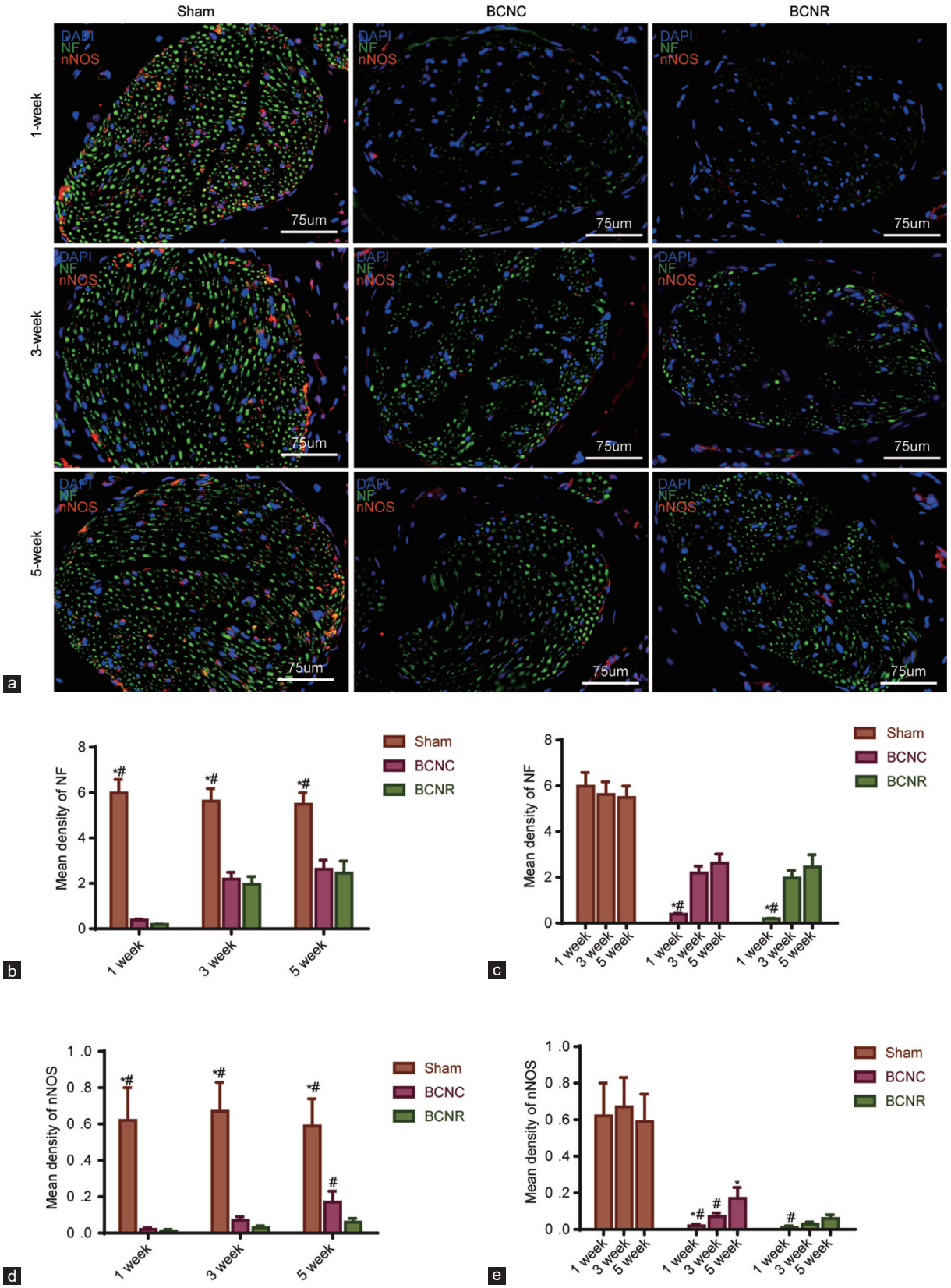
Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

REFERENCES

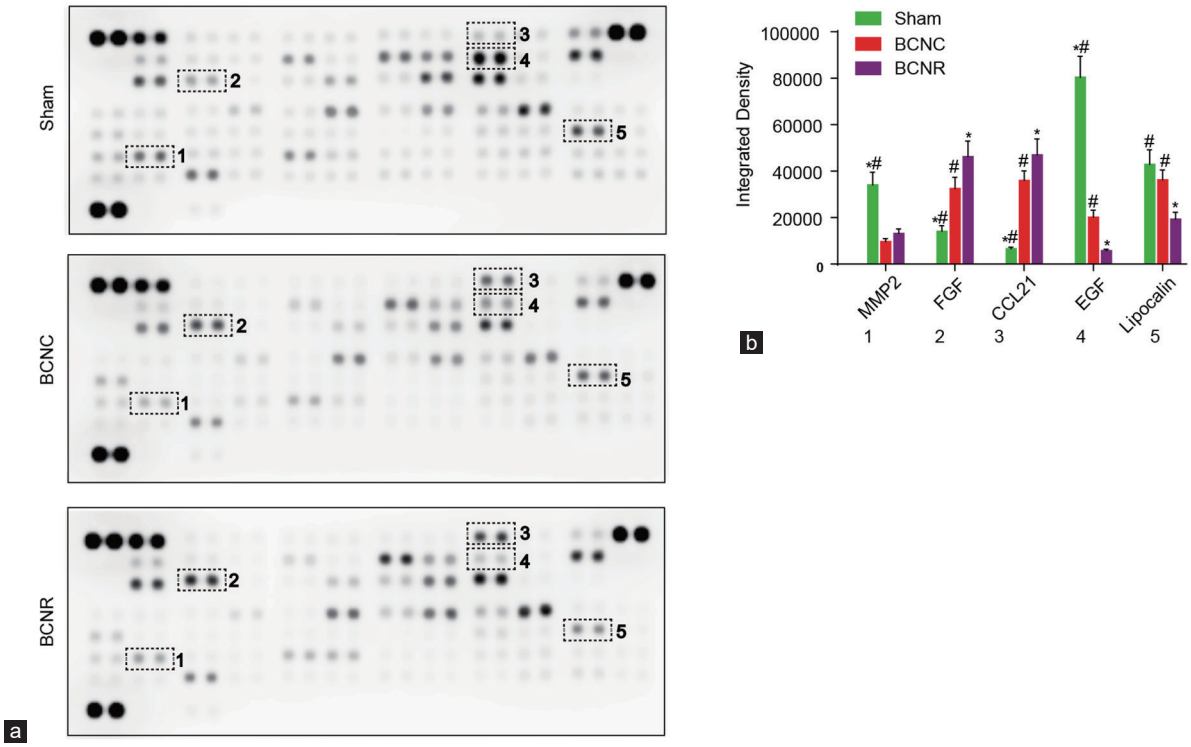
1 Walsh PC, Donker PJ. Impotence following radical prostatectomy: insight into etiology and prevention. *J Urol* 2017; 197: S165–70.
2 Kendirci M, Hellstrom WJ. Current concepts in the management of erectile dysfunction in men with prostate cancer. *Clin Prostate Cancer* 2004; 3: 87–92.
3 Awad A, Alsaid B, Bessede T, Droupy S, Benoît G. Evolution in the concept of erection anatomy. *Surg Radiol Anat* 2011; 33: 301–12.
4 Leungwattanakij S, Bivalacqua TJ, Usta MF, Yang DY, Hyun JS, *et al*. Cavernous neurotomy causes hypoxia and fibrosis in rat corpus cavernosum. *J Androl* 2003; 24: 239–45.
5 Ferrini MG, Kovanecz I, Sanchez S, Umeh C, Rajfer J, *et al*. Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. *J Sex Med* 2009; 6: 415–28.
6 Yiou R. Stem-cell therapy for erectile dysfunction. *Biomed Mater Eng* 2017; 28: S81–5.
7 Wu C, Kovac JR. Models for erectile dysfunction and their importance to novel drug discovery. *Expert Opin Drug Discov* 2016; 11: 185–96.
8 Weyne E, Mulhall J, Albersen M. Molecular pathophysiology of cavernous nerve injury and identification of strategies for nerve function recovery after radical prostatectomy. *Curr Drug Targets* 2015; 16: 459–73.
9 Chung E, De Young L, Brock GB. Investigative models in erectile dysfunction: a state-of-the-art review of current animal models. *J Sex Med* 2011; 8: 3291–305.
10 Kapoor MS, Khan SA, Gupta SK, Choudhary R, Bodakhe SH. Animal models of erectile dysfunction. *J Pharmacol Toxicol Methods* 2015; 76: 43–54.
11 Haney NM, Nguyen HM, Honda M, Abdel Mageed AB, Hellstrom WJ. Bilateral cavernous nerve crush injury in the rat model: a comparative review of pharmacologic interventions. *Sex Med Rev* 2018; 6: 234–41.
12 Hannan JL, Kutlu O, Stopak BL, Liu X, Castiglione F, *et al*. Valproic acid prevents penile fibrosis and erectile dysfunction in cavernous nerve-injured rats. *J Sex Med* 2014; 11: 1442–51.
13 Canguven O, Lagoda G, Sezen SF, Burnett AL. Losartan preserves erectile function after bilateral cavernous nerve injury via antifibrotic mechanisms in male rats. *J Urol* 2009; 181: 2816–22.
14 Kendirci M, Zsengellér Z, Bivalacqua TJ, Gur S, Usta MF, *et al*. Poly(adenosine diphosphate-ribose) polymerase inhibition preserves erectile function in rats after cavernous nerve injury. *J Urol* 2005; 174: 2054–9.
15 Ferrini MG, Davila HH, Kovanecz I, Sanchez SP, Gonzalez-Cadavid NF, *et al*. Vardenafil prevents fibrosis and loss of corporal smooth muscle that occurs after bilateral cavernosal nerve resection in the rat. *Urology* 2006; 68: 429–35.
16 Li M, Lei H, Xu Y, Li H, Yang B, *et al*. Exosomes derived from mesenchymal stem cells exert therapeutic effect in a rat model of cavernous nerves injury. *Andrology* 2018; 6: 927–35.
17 Andersson KE. Erectile physiological and pathophysiological pathways involved in erectile dysfunction. *J Urol*. 2003; 170: S6–14.
18 Dean RC, Lue TF. Physiology of penile erection and pathophysiology of erectile dysfunction. *Urol Clin North Am* 2005; 32: 379–95,v.
19 van Driel MF. Physiology of penile erection-a brief history of the scientific understanding up till the eighties of the 20th century. *Sex Med* 2015; 3: 349–57.
20 Yang F, Zhao JF, Shou QY, Huang XJ, Chen G, *et al*. Phenotypic modulation of corpus cavernosum smooth muscle cells in a rat model of cavernous neurectomy. *PLoS One* 2014; 9: e105186.
21 Lin G, Li H, Zhang X, Wang J, Zaid U, *et al*. Novel therapeutic approach for neurogenic erectile dysfunction: effect of neurotrophic tyrosine kinase receptor type 1 monoclonal antibody. *Eur Urol* 2015; 67: 716–26.
22 Prudovsky I, Mandinova A, Soldi R, Bagala C, Graziani I, *et al*. The non-classical export routes: FGF1 and IL-1alpha point the way. *J Cell Sci* 2003; 116: 4871–81.
23 Jin HR, Chung YG, Kim WJ, Zhang LW, Piao S, *et al*. A mouse model of cavernous nerve injury-induced erectile dysfunction: functional and morphological characterization of the corpus cavernosum. *J Sex Med* 2010; 7: 3351–64.
24 Kutlu O, Karaguzel E, Okatan AE, Mentese A, Yulug E, *et al*. Dipyridamole reduces penile apoptosis in a rat model of post-prostatectomy erectile dysfunction. *Int Braz J Urol* 2017; 43: 966–73.
25 Grinman DY, Romorini L, Presman DM, Rocha Viegas L, Coso OA, *et al*. Role of 3'-5'-cyclic adenosine monophosphate on the epidermal growth factor dependent survival in mammary epithelial cells. *Mol Cell Endocrinol* 2016; 419: 259–67.



Supplementary Figure 1: Changes in nNOS and NF medium-positive neurons in the major pelvic ganglion (MPG) at 1 week, 3 weeks, and 5 weeks after bilateral cavernous injury. (**a**) Representative immunofluorescence images of nNOS (red) and NF (green) in the MPG at ×400. (**b**) Comparison of semi-quantitative analysis of NF expression between treatment groups at each time point. $^{*}P < 0.05$ means BCNR and Sham versus the BCNC group, $^{\#}P < 0.05$ means BCNC and Sham versus the BCNR group. (**c**) Comparison of NF expression within treatment groups at each time point. $^{*}P < 0.05$ means week 1 and week 5 versus week 3, $^{\#}P < 0.05$ means week 1 and week 3 versus week 5. (**d**) Comparison of semi-quantitative analysis of nNOS expression between treatment groups at each time point. $^{*}P < 0.05$ means BCNR and Sham versus the BCNC group, $^{\#}P < 0.05$ means BCNC and Sham versus the BCNR group. (**e**) Comparison of nNOS expression within treatment groups at each time point. $^{*}P < 0.05$ means week 1 and week 5 versus week 3, $^{\#}P < 0.05$ means week 1 and week 3 versus week 5. nNOS: neuronal-nitric oxide synthase; NF: neurofilament.



Supplementary Figure 2: Changes in nNOS and NF medium-positive neurons in the penile dorsal nerve (PDN) at 1 week, 3 weeks, and 5 weeks after bilateral cavernous injury. (**a**) Representative immunofluorescence images of nNOS (red) and NF (green) in the PDN at ×400. (**b**) Comparison of semi-quantitative analysis of NF expression between treatment groups at each time point. $^{*}P < 0.05$ means BCNR and Sham versus the BCNC group, $^{\#}P < 0.05$ means BCNC and Sham versus the BCNR group. (**c**) Comparison of NF expression within treatment groups at each time point. $^{*}P < 0.05$ means week 1 and week 5 versus week 3, $^{\#}P < 0.05$ means week 1 and week 3 versus week 5. (**d**) Comparison of semi-quantitative analysis of nNOS expression between treatment groups at each time point. $^{*}P < 0.05$ means BCNR and Sham versus the BCNC group, $^{\#}P < 0.05$ means BCNC and Sham versus the BCNR group. (**e**) Comparison of nNOS expression within treatment groups at each time point. $^{*}P < 0.05$ means week 1 and week 5 versus week 3, $^{\#}P < 0.05$ means week 1 and week 3 versus week 5. nNOS: neuronal-nitric oxide synthase; NF: neurofilament.



Supplementary Figure 3: Cytokine arrays of protein extracted from penile tissue at week 3 after bilateral cavernous injury. (**a**) Cytokine array of penis proteins. (**b**) Comparative analysis of protein expression between treatment groups. $^{*}P < 0.05$ means and Sham versus the BCNC group, $^{\#}P < 0.05$ means BCNC and Sham versus the BCNR group. MMP-2: matrix metalloprotein-2; FGF: fibroblast growth factor; CCL21/SLC: C-C motif ligand 21/fibroblast chemotactic factor; EGF: epidermal growth factor; BCNR: bilateral cavernous nerve resection; BCNC: bilateral cavernous nerve crushing.