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Brief communication (Original)

Effect of sacral nerve electrostimulation on sex dysfunction in male rats with spinal cord injury

Jiawen Wu[a, b], Jianzhong Hu[a], Hongbin Lu[c], Qi Liao[b], Juanjuan Wang[d], Mingxin Wu[e], Chunhan Sun[f], Ming Kang[e]

[a]Department of Spinal Surgery, Xiangya Hospital, Central South University, Hunan 410008, [b]Department of Orthopedics, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, [c]Department of Sport Medicine, Xiangya Hospital, Central South University, Hunan 410008, [d]Department of Ophthalmology, The Second Affiliated Hospital Of Nanchang University, Nanchang 330006, [e]Department of Orthopedics, Huizhou Municipal Central Hospital, [f]Department of Orthopedics, The First People·s Hospital Of Huizhou, Guangdong 516001, China

Background: Spinal cord injury severely impairs fertility of young men. It can induce erectile dysfunction, ejaculatory dysfunction, and semen abnormalities. However, the precise mechanism is unclear.

Objective: We observed the effects of sacral nerve electrostimulation on sex dysfunction of male rats with spinal cord injury.

Methods: Thirty-six male Sprague-Dawley (SD) rats were randomly divided into three groups (n = 12): sham operated group, spinal cord injury model group, and electrostimulation group. The weight of genitals, related sex hormone index, and sperm motility were examined in each group after operation at two, four, and six weeks, respectively.

Results: Sacral nerve electrostimulation could improve testis and epididymis weight, regulate hormone secretion (including an increase in GnRH, tT, and ABP, but decrease in FSH and LH), and cause an increase in sperm count and motility.

Conclusion: Sacral nerve electrostimulation may be effective in treating sex dysfunction resulting from spinal cord injury.

Keywords: Hormone, sacral nerve electrostimulation, sexual dysfunction, sperm, spinal cord injury

Spinal cord injury (SCI) occurs most often in young men at the peak of reproductive health [1]. After SCI, fertility in most men is severely impaired due to erectile dysfunction, ejaculatory dysfunction, and semen abnormalities [2].

The sacral segments S2–S4 contribute innervation to the penis, and have a role in controlling reflexogenic erections. In the sacral area, postganglionic fibers from the sympathetic chain ganglia pass to the sacral nerves and then to the pelvic or pudendal nerves. Parasympathetic preganglionic axons pass via the pelvic nerve to the pelvic plexus. Ganglion cells in the pelvic plexus send axons into the cavernous nerve, which innervates the penis. The pudendal nerve originates in the S2 to S4 segments of the spinal cord and innervates the external sphincter, the bulbospongiosus muscle and the ischiocavernosus muscle. Thereby, pudendal nerve also provides sensory fibers to the dorsal nerve of the penis [3]. Thus, electrical stimulation of the sacral nerve, pelvic nerve, sympathetic stimulation, and perineal could benefit erectile dysfunction. For example, it was known that electrical stimulation of the pelvic nerve and sympathetic nerve can mediate erections [4]. Study also indicated 29 of 33 subjects could achieve a full sustainable erection by stimulating the S2 or S3 anterior routes [5]. Perineum electrostimulation could also induce erection by affecting intra-corporeal pressure increase [6]. Cavernous nerve electrostimulation results in an erection by causing increased arterial flow, relaxation of the cavernous muscles, and venous outflow restriction [7]. In addition, electroejaculation and penile vibratory stimulation have been used to induce ejaculation [8].

Correspondence to: Jianzhong Hu, Department of Spinal Surgery, Xiangya Hospital, Central South University, Hunan 410008, China. E-mail: hjz3734@vip.sina.com

However, the detailed physiology mechanism of electrostimulation is still unclear. The weight of genitals and endocrine profiles have been studied associated with semen quality of SCI men, such as with low gonadotropins [9], normal, low, or high testosterone [10], normal, low or high follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and normal or high prolactin [11, 12]. In this study, our aim is to study the effect of sacral nerve electrostimulation on sexual dysfunction through the changes of genital weights, related sex hormone index, and sperm motility.

Material and methods

Preparing spinal cord injury rats

Thirty-six healthy male Sprague-Dawley (SD) rats weighting 250 to 275g were randomly divided into three groups (n = 12): sham operated group (A), spinal cord injury model group (B), and electrostimulation group (C). For a sham control, only a skin incision was at T9-T10. Spinal cord injury model was established by complete laminectomy and Allen's weight drop at the T9-T10 spinal level. Further bilateral stimulation of the S3 nerve was performed in electrostimulation group operating at 3 volt, 20 Hz, and continuous stimulating for 8 hours everyday. After injury, the weight of genitals, related sex hormone index, and sperm motility were determined in each group after operation of two, four, and six weeks, respectively.

The study is approved by the Animal Care and Use Committee of the Xiangya Hospital, Central South University.

Pathological analysis

The pathological changes of testis, prostate, and epididymis were examined using Hematoxylin-Eosin (HE) and toluidine blue staining. The sample (<0.5cm dimensions) was dissected and fixed with Bouin's fluid. Then dehydrated in a graded alcohol series and embedded in paraffin. The thin sections were stained with HE followed by a toluidine blue. Images were captured using an Olympus BX51 microscope.

Hormone measurement

Gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (tT), and androgen binding protein (ABP) concentrations were determined with a commercially available enzyme-linked immunosorbent assay kit (ELISA) (R&D Systems, USA).

Sperm motility

The epididymides were dissected immediately and immersed in 4 mL of Ringer solution. The distal end of the caudal epididymis was and the sperm were flushed out with buffer. The sperm suspension was kept at 35°C for 10 minutes [13]. Sperm was diluted by adding another 8 ml normal saline. Motility was scored in cell counting chamber (× 400). More than 200 sperm were examined from each animal within 10 minutes. Sperm motility was classified into four grades, namely; a) rapid linear progressive motility, b) slow or sluggish linear or non-linear progressive motility, c) non-progressive motility (also called motility on the spot), and d) immotile [14, 15]. The total count from diluted semen samples was computed using a Hemacytometer chamber [16].

Statistical analysis

All data were analyzed by SPSS17.0 and the results were measured by average±standard deviation ($\bar{x}$±s). Statistical analysis was carried out using the unpaired Student's t-test or one-way analysis of variance (ANOVA). p <0.05 was considered as statistically significant.

Results

Pathological analysis of testis

Few changes were observed in sham treatment group (**Figure 1A**), Leydig cells in connective-tissue between seminiferous tubules were still large, their cytoplasm showed acidophily, and the nucleus was round or oval in shape.There was a distinct stratification of spermatogenic cells in convoluted seminiferous tubules at different developmental stage, including spermatogonium, primary spermatocyte, secondary spermatocyte, and spermatoblast. Mature sperm passed into the lumina of the seminiferous tubules and left the testis. The testis is covered by an external tunica serosa and internal tunica albuginea made up of dense connective-tissue. However, SCI (**Figure 1B**) led to hemangiectasis, hyperaemia, inflammatory cells infiltration, and edema in Leydig cells. Disordered arrangement of spermatogenic cells appeared in convoluted seminiferous tubules. Few mature sperm were seen within the testis. Sacral nerve electrostimulation(**Figure 1C**) caused an improvement in testis structure, such as hyperaemia, inflammatory cell infiltration, and edema gradually disappeared in Leydig cells, stratification of spermatogenic cell was restored, especially after four and six weeks. Total mature sperm also increased.

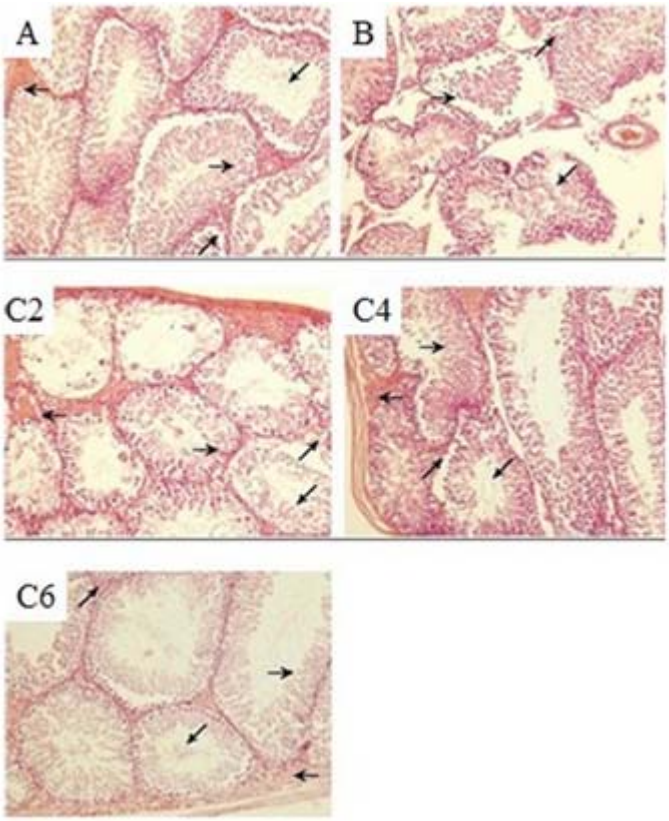

Figure 1. Sacral nerve electrostimulation weaken SCI's damage to testis SCI led to hemangiectasis, hyperaemia, inflammatory cells infiltration, and edema in leydig cell (B), sacral nerve electrostimulation caused an improvement in testis structure (C). **A:** group A, **B:** group B, **C2:** group C, two weeks, **C4:** group C, four weeks, **C6:** group C, six weeks, ↑ = leydig cells, → = spermatogenic cell, ↓ = mature sperm, ← = tunica serosa and tunica albuginea

Pathological changes of rat prostates and epididymis

Prostate is a compound tubularacinous gland, with irregular prostatic vesicles. Glandular epithelium could be classified into simple cuboidal epithelium, simple columnar epithelium, and pseudostratified ciliated columnar epithelium. Few loose connective tissue and smooth muscle cells are present in mesenchyme (**Figure 2A**). Epididymis wall is made up of pseudostratified ciliated columnar epithelium, with granules in cytoplasm, amount of mature sperm in epididymis lumen (**Figure 3A**). Our results also indicated that SCI (**Figures 2B, 3B**) led to hyperplasia in glandular and mesenchyme, and concomitant with hyperaemia, edema, and neutrophil infiltration. Sacral nerve electrostimulation (**Figures 2C, 3C**) seemed to improve this in mesenchyme. Hyperplasia was reduced, hyperaemia, inflammatory cells infiltration, and edema gradually decreased, especially after four and six weeks. The number of mature sperm was also reduced (**Figure 3B**).

Sacral nerve electrostimulation of the organ weight

Two weeks after spinal cord injury, unilateral testis and epididymis weight increased compared to that of the sham-operated group, but prostate weight decreased. However, after four and six weeks, there was a significant decrease in unilateral testis [17] and epididymis weight compared to that of sham-operated group, but an increase in prostate weight. Sacral nerve electrostimulation induced an improvement from the above changes. That is, unilateral testis and epididymis weight were reduced compared to SCI group. Four and six weeks later, a significant increase was observed in unilateral testis and epididymis weight compared to SCI group. Yet, prostate weight was found to rise after two, four and six week of sacral nerve electrostimulation, indicating sacral nerve electrostimulation had little effect on prostate, but more on testis and epididymis.

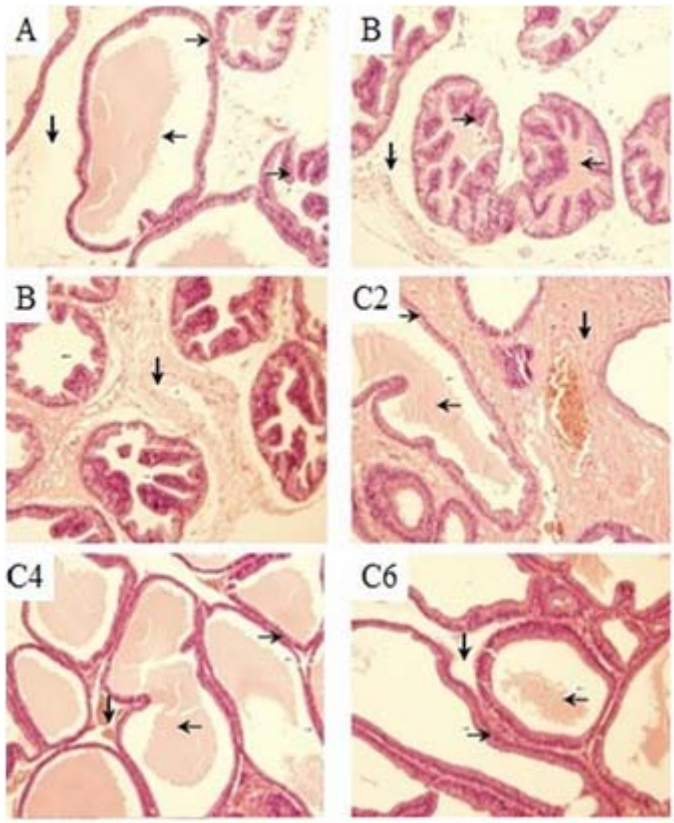

Figure 2. Sacral nerve electrostimulation pathological changes of prostate. SCI led to hyperplasia in glandular and mesenchyme, and concomitant with hyperaemia (B), while sacral nerve electrostimulation seemed to improve above symptoms in mesenchyme (C). **A:** group A, **B:** group B, **C2:** group C, two weeks, **C4:** group C, four weeks, **C6:** group C, six weeks, ← = secretion in glandular cavity, → = glandular epithelium, ↓ = loose connective tissue and smooth muscle

Hormone analysis

From our results, we found that GnRH, tT, and ABP content significantly decreased in group B compared with group A, but showed an increase by sacral nerve electrostimulation in group C, especially after four and six weeks. Conversely, FSH and LH content significantly increased in group B compared with groupA, but showed a decrease by sacral nerve electrostimulation in group C. Additionally, this tendency was similar after two, four, and six weeks. These results indicated that a synergistic relationship was present among GnRH, tT, and ABP, FSH and LH. Antagonistic relationship was present between GnRH, tT, and ABP, FSH and LH.

Sacral nerve electrostimulation increase sperm motility

Sperm motility is essential for normal fertilization, and asthenozoospermia, or low sperm motility, is common in infertile men [18]. According to our results, sperm count significantly decreased after SCI, especially after four and six weeks. Additionally, the sperm motility was gradually abolished, showing more sperm was classified into II, III, and even IV grade. However, sacral nerve electrostimulation treatment led to an improvement of sperm count and motility, namely, significantly increase in sperm count, and sperm motility of I and II grade.

The effect of rat nerve stimulation on organs weight and motility

Our findings demonstrated that SCI had a significant effect on testis, epididymis, and prostate (**Table 1**), all resulting in hemangiectasis, hyperaemia, inflammatory cells infiltration, and edema of mesenchymal cells. Importantly, SCI led to a decrease in mature sperm amount in testis and epididymis (**Table 2**). Fewer sperm would result in a decrease in testis and epididymis weight. However, an increase in prostate weight was suggested by hyperplasia in glandular and mesenchyme.

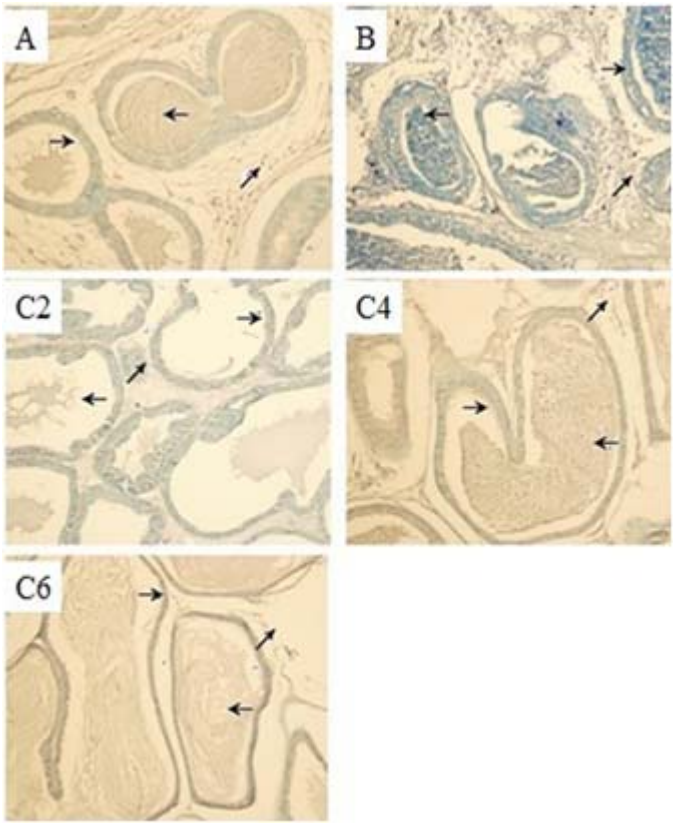

Figure 3. Sacral nerve electrostimulation pathological changes of epididymis. It indicated SCI led to edema and neutrophil infiltration (Fig 3B). Sacral nerve electrostimulation(Fig 2C, 3C) seemed to decrease edema gradually, especially after four and six weeks. **A:** group A, **B:** group B, **C2:** group C, two weeks, **C4:** group C, four weeks, **C6:** group C, six weeks, ← = mature sperm, → = glandular epithelium, ↗ = mesenchyme

Table 1. The effect of sacral nerve electrostimulation on organs weight of SCI rat

		Unilateral testis (g)	Unilateral epididymis (g)	Prostate (g)
	Two weeks	1.96 ± 0.15	0.26 ± 0.03	0.44 ± 0.19
A	Four weeks	2.73 ± 0.56	0.33 ± 0.06	0.27 ± 0.08
	Six weeks	2.73 ± 0.21	0.26 ± 0.07	0.26 ± 0.06
	Two week	2.40 ± 0.66	0.31 ± 0.14	0.31 ± 0.11
B	Four weeks	1.43 ± 0.46	0.25 ± 0.09	0.41± 0.14
	Six weeks	1.78 ± 0.10	0.2 ± 0.04	0.28 ± 0.03
	Two weeks	2.19 ± 0.61	0.26 ± 0.07	0.43 ± 0.21
C	Four weeks	1.64 ± 0.21	0.27 ± 0.05	0.51 ± 0.19
	Six weeks	1.52 ± 0.08	0.26 ± 0.05	0.29 ± 0.03

Table 2. The effect of sacral nerve electrostimulation on sperm motility of SCI rat

		Count	Motility
A	Two weeks	7.48 ± 1.46	I (4)
	Four weeks	6.21 ± 1.94	I (4)
	Six weeks	5.98 ± 0.91	I (4)
B	Two weeks	2.15 ± 3.19	II (2), III (2)
	Four weeks	0.09 ± 0.09	II (1), III (2), IV (1)
	Six weeks	0.15 ± 0.07	II (2), III (1)
C	Two weeks	0.89 ± 0.23	I (1), II (3)
	Four weeks	1.95 ± 1.15	I (3), II (1)
	Six weeks	2.33 ± 2.25	I (3), II (1)

Effect on hormone regulation

Therefore, our results show a change in endocrine profiles, exhibiting a decrease in GnRH, tT, and ABP content, but increase in FSH and LH content when subjected by SCI (**Table 3**).

Discussions

We showed that SCI had significant effect on testis, epididymis, prostate, sperm count, and hormone regulation. Mammalian spermatogenesis is a fine tuned process by hormone regulation [19]. This was in accordance with our previous study in humans. For example, there were also eight SCI patients who had low serum tT and eight SCI patients with elevated FSH and/or LH in [20]. The elevated serum FSH levels were consistent with impaired spermatogenesis frequently seen in SCI males [21]. Subjects who had elevated FSH were azoospermic [22]. Reductions in tT and sex hormone-binding globulin (SHBG) levels are associated with complaints of erectile dysfunction [23]. Serum LH levels were significantly higher in both subgroups than in the control group. Free tT levels were lower in the subgroup after injury d”12 months than in the other patient subgroup and the control group [24].

Our previous studies found that the detrimental effects of SCI on spermatogenesis in the rat can be attenuated by exogenous tT but enhanced by exogenous FSH [25, 26]. Implantation of 3 to 20-cm testosterone-filled Silastic capsules (TCs) resulted in dose-dependent increases of serum tT levels in SCI. Serum LH and FSH concentrations of SCI rats decreased in a dose-dependent manner in those that received TC implants [27]. These reports demonstrated that there was an antagonism relationship between tT and FSH and LH. Our work also indirectly demonstrated this conclusion. Therefore, sacral nerve electrostimulation induced more tT to negatively regulate the release of FSH and LH.

Table 3. The effect of sacral nerve electrostimulation on hormone secretion of SCI rat

		A	B	C
GnRH	Two weeks	2329.79 ± 725.98	1287.77 ± 491.82	1646.95 ± 363.32
	Four weeks	1787.21 ± 352.08	1094.44 ± 364.17	1818.16 ± 840.24
	Six weeks	1818.53 ± 525.85	1242.87 ± 324.03	1607.19 ± 337.53
FSH	Two weeks	2.34 ± 0.77	5.48 ± 1.67	2.65 ± 0.38
	Four weeks	2.09 ± 0.91	3.90 ± 0.84	1.67± 0.48
	Six weeks	1.74 ± 0.42	3.71 ± 1.02	1.35 ± 0.13
LH	Two weeks	265.14 ± 49.85	477.51 ± 99.97	367.65 ± 157.23
	Four weeks	255.20 ± 48.94	427.97 ± 150.51	294.85 ± 62.62
	Six weeks	272.10 ± 81.94	530.09 ± 52.12	279.63 ± 26.67
tT	Two weeks	9.76 ± 3.55	4.17 ± 1.76	3.51 ± 1.86
	Four weeks	7.64 ± 5.63	4.87 ± 2.75	7.70 ± 2.82
	Six weeks	8.35 ± 4.41	2.60 ± 1.11	6.61 ± 2.27
ABP	Two weeks	2067.40 ± 437.44	1734.29 ± 178.41	1304.65 ± 385.44
	Four weeks	1889.46 ± 475.32	1705.20 ± 211.11	1248.42 ± 497.85
	Six weeks	2418.83 ± 413.90	1328.80 ± 351.20	1987.89 ± 468.26

However, this suppression effect of tT administration to both LH and FSH secretion could be blocked by a GnRH agonist indicating synergism relationship between tT and GnRH [28]. After withdrawal of the GnRH antagonist and on restoration of normal serum tT concentrations, these erectile abnormalities were reversed [29]. Our study also reflected this conclusion, namely, tT, and GnRH content decreased in SCI patients, but increased after sacral nerve electrostimulation.

Conclusion

We suggest that sacral nerve electrostimulation could improve testis and epididymis weight, regulate hormone secretion (including an increase in GnRH, tT, and ABP, decrease in FSH and LH), and cause increase in sperm count and motility.

The authors have no conflict of interest to report.

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