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## **Validation of a modified rat model for erectile function evaluation**

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## Abstract

The in vivo model for evaluation of erectile function in rats and mice has been widely used to investigate pathophysiology and treatment modalities of erectile function. The model is technically challenging which limits its broad availability. We have recently introduced a simplified surgical technique for dissection of corporal bodies and developed a new method to achieve stable contact between the cavernous nerve and the stimulating electrode without the need to manipulate the nerve between stimulations using 2-component silicone glue. The goal of this study was to validate this new technique and describe in detail the technical aspects of the procedure so that researchers with basic microsurgery skills can adopt it.

## Introduction

Erectile dysfunction (ED) affects up to 52% of men aged 40 – 70 (1). Its incidence has been increasing over the last decades, and it has been estimated that it might affect up to 150 million men worldwide (2). The pathophysiology of ED can be vasculogenic, neurogenic, anatomical, hormonal, drug-induced, and/or psychogenic (3). One common iatrogenic cause of erectile dysfunction is radical prostatectomy, and the reported rate of postoperative erectile dysfunction ranges from 14 - 90% (4-6). The first line treatment of ED is phosphodiesterase type 5 (PDE5i) therapy, however it has been reported to have up to 35% failure rate, thus prompting further research in this rapidly growing field (7). Recent studies have explored the use of autologous stem cell transplantation as a unique alternative (8).

A large proportion of the existing knowledge regarding erection pathophysiology and treatment modalities has been obtained with the use of animal models. Large animals were used between the 60's and the 90's, including cats, dogs, and monkeys. Since introduction of the rat model for the study of penile erection by Quinlan et al., rodents have largely replaced other animals. Quinlan et al. first demonstrated that electrical stimulation of the cavernous nerve in Sprague-Dawley rats resulted in reproducible tumescence of the corpora cavernosum. Since that time, the model has been widely adopted and modified. Search term "animal model of erection" applied in PubMed yielded 40 of publications in 2020. Of those, 26 papers used rat model, 4 papers used mouse and 0 papers used large animals. Cavernous nerve (CN) stimulation and intracavernous pressure (ICP) recording was used in 16 papers, and other less precise methods, such as visual scoring of penile erection, were used in 2 papers. In 2 papers, no in vivo erectile function assessment was used at all. When reviewing these manuscripts, it became obvious that in vivo erectile function evaluation, using recording of ICP increase during pharmacological or CN stimulation would have been of value. We believe that the reason for this is that although reliable, the rat model using electrostimulation of CN and recording of the pressure in the cavernous bodies of the penis is

technically challenging and difficult to adopt for teams without a member trained in this technique. Therefore, we developed microsurgical technique which simplifies the procedure. The goal of this communication is to describe the procedure in detail and provide data validating the reproducibility of this modified model of bilateral CN crush injury.

## Material and Methods

Twenty-four male 10 – 12 weeks old Sprague-Dawley rats (weighed 260 – 330g) were housed at the University of Southern Denmark Central Animal Care Facility as per institutional guidelines. The Ethics Committee of the Danish Animal Experiments Inspectorate approved the study procedures. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were split into three study groups (Figure 1).

### 1. First Procedure: Bilateral Nerve Crush

#### 1.1 Nerve crush

Each animal was initially anesthetized with subcutaneous fentanyl (235 mg/kg), fluanisone (7.5 mg/kg) and midazolam (3.75 mg/kg). The neck and lower abdomen were shaved and cleaned with 70% alcohol. The rat was then placed supine on a heated surgical pad and oxygen was supplied through the nose cone at a rate 0.8 L/min. Depth of anesthesia was tested with toe pinch. Next, a 2 cm vertical midline abdominal incision was made using a scalpel. After dissecting through the abdominal wall, the bladder was identified, decompressed, and retracted laterally and cranially. Dissection using cotton-tip swabs (Q-tips) was used to expose major pelvic ganglion (MPG) and the CN running vertically on the dorsolateral aspect of the prostate (Figure 2). The cavernous nerve was freed from the tissue around 3 – 4 mm distal from the MPG. A micro needle holder was then placed on the nerve and clamped. Crush injury was induced with clamping the nerve for two minutes using micro needle holder. This process was repeated bilaterally. The abdominal wall layers were closed with a running suture. The animal was rehydrated with bolus saline and left to

recover under a heat lamp with temperature monitoring. Pain control was achieved with buprenorphine.

## **2. Second Procedure: CN Stimulation and ICP Measurement**

### **2.1 Carotid Cannulation for MAP Measurement**

The carotid artery was used for blood pressure monitoring for the duration of surgery. A 1.5 cm vertical midline incision was made on the neck above the sternal notch. Muscular and fascial planes were dissected away to the level of the carotid artery. The carotid was cannulated with a polyethylene (PE) 50 tubing which was secured in place with three 4-0 silk sutures. The tubing was connected to a pressure transducer (Digitimer/NeuroLog, Cambridge, UK) and zeroed to the level of the artery.

### **2.2 Dissection of the Cavernous Body**

A 1 centimeter vertical skin incision was made 5 mm lateral to the midline starting at the level of the base of the penis and extending downward. Using palpation of the ischial tuberosity (point of penile crus insertion) as an anatomical landmark, the ischiocavernosus muscle was exposed using blunt dissection with Q-tips (Figure 3). Using curved microforceps, the muscle fibers were spread longitudinally until exposing the tunica albuginea which appears bright white (Figure 4). A 5 mm incision was made through the skin lateral to the penile crus exposure. After pressure calibration, the PE50 tubing was passed through the skin incision parallel to the crus and connected to a 23G gauge needle (Figure 5). At this point, cannulation was paused until after placement of the electrode on the cavernous nerve.

### **2.3 Electrode Placement**

Through a 2 cm long lower, midline abdominal incision, the lateral aspect of the prostate was exposed. Using blunt dissection with Q-tips, the MPG and CN were visualized. Micro scissors were used to incise the fascia overlying the nerve on each side and a 9-0 nylon suture was slid underneath

the 0.3 mm section of CN freed from the underlying tissue (Figure 6). The CN was lifted and a 125  $\mu$ m silver bipolar electrode was placed underneath. A micromanipulator was used to hold the electrode. After lifting the nerve slightly, so that the neither the nerve or the electrode were touching the surrounding tissue, the electrode was dried and biocompatible silicone glue (Kwik-Sil, WPI, Sarasota, FL) was applied (Figure 7). After keeping the nerve elevated for 2 – 3 minutes (time necessary for the glue to dry) a safe contact between the electrodes and the nerve was achieved. The nerve was then returned to its normal position and no other manipulation was needed. At this point, the abdominal incision was filled with 0.9% saline.

#### 2.4 Cavernous Body Cannulation

At the surgical site lateral to the scrotum, the line was filled with heparinized 0.9% NaCl connected to the 23G needle was inserted into the crus of the penis 2 - 3 mm proximal from the ischial tuberosity, pointing toward the tip of the penis (Figure 8).

#### 2.5 CN stimulation and ICP recording

Electrical stimulations were delivered with Square Pulse Stimulator SD9K (Astro-Med Grass Technologies, AstroNova, West Warwick, RI). Stimuli were made with a current at 1.5 mA, frequency of 16 Hz, a voltage of 3 V, and pulse width at 5 ms. The stimulations lasted 50 s, with 1 minute rest between the stimuli. Stimulation was repeated until three equal responses were recorded. ICP recording was performed in three groups of rats (n=8 per group) including healthy controls, and animals two and four weeks after CN injury.

#### Statistical considerations

The values were reported as the mean and standard error of the mean. A two-sample t-test was used to compare peak and mean ICP increase and area under the pressure curve during CN stimulation in control healthy animals and animals two and for weeks after the CN injury. The differences between groups were considered statistically significant at p-value < 0.05.

## Results

Isolation of the nerve-electrode complex from the surrounding soft tissue with silicon glue made it possible to obtain reproducible pressure recordings in all 24 rats. The ICP increase during CN stimulation was compared between the intact healthy animals and rats two and four weeks after the CN injury. Each group included eight rats. For rats in the control group, the peak ICP during CN stimulation was  $82 \pm 4$  and the mean ICP was  $71 \pm 5$ . For rats two weeks after CN injury, the peak ICP was  $53 \pm 4$  mmHg and the mean ICP was  $41 \pm 4$  mmHg. For rats in four weeks after CN injury, the peak ICP was  $57 \pm 3$  mmHg and the mean ICP was  $39 \pm 2$  mmHg (Figure 9). We also analyzed the area under the curve as an indicator of total ICP generated over the entire time course of stimulation. The area under the curve was  $39.4 \pm 2.3$  in the control group and  $22.7 \pm 1.5$  and  $21.9 \pm 1.2$  in the animals two and four weeks post CN crush injury (Figure 10).

## Discussion

The rat model of erectile dysfunction initially described by Quinlan et al. in 1989 has been successfully used for decades with slight modifications over time by a number of researchers (9). Sprague Dawley rats, ages 6 - 12 weeks old, have been used in the majority of studies. Certain aspects of this model have been outlined in detail, such as nerve dissection and method of nerve stimulation (10, 11). Other aspects of the procedure are frequently not described and there is concern that they vary amongst studies. It is uncertain which modifications improve reproducibility and which simply make the surgery easier for the individual researcher (12). We believe that main pitfalls of the current rat model include inconsistent method for cannulation of the cavernous bodies and lack of a stable contact between the nerve and the electrode during electrostimulation (12).

Many access the erectile bodies for cannulation distal to the base of the penis using de-gloving. This method of dissection can injure structures which are important for regulation of erectile function. In addition, inserting the needle into the external part of the penis (which moves during



erection) makes the cannulation unstable. In this study, we accessed the cavernous tissue by cannulating the penile crus lateral to the scrotum, close to the point of its insertion to the ischial tuberosity. After performing the skin incision, the ischial tuberosity can be palpated and used as landmark, simplifying the identification of penile crus. In addition, dissection in this area preserves the neural and vascular structures. Introducing the needle through the skin lateral to the crus keeps it parallel with the crus, allowing for a stable position.

An additional source of inconsistent recordings is CN stimulation. The Quinlan technique uses rather big platinum bipolar hook electrodes and requires lifting and drying the nerve prior to each stimulation. The repetitive stretching and touching of the nerve can cause nerve damage. In addition, if the nerve is not adequately isolated, electrical current can leak out to surrounding tissues. This results in either falsely decreased ICP measurements, or falsely elevated ICP measurements if the ischiocavernosus muscle is accidentally stimulated. We have addressed this issue by using a much smaller bipolar Teflon-coated silver electrode (125  $\mu\text{m}$  in diameter) and the two-component biocompatible silicone glue to isolate and stabilize the nerve-electrode complex for repetitive stimulation. This method eliminates any manipulation of the nerve between stimulations and allows for reproducible recordings.

Previous studies have measured ICP between 10 days to eight weeks after nerve crush injury, with most using four weeks interval (13-17). To our knowledge, no studies compared function after different time intervals. During the first two weeks after nerve crush injury, the animal completely recovers from the surgery, and no alterations to its physiological function are expected, however CN may undergo spontaneous regeneration. We therefore included both two and four-week interval between nerve crush injury and erectile function evaluation. We showed consistent reduction in erectile response after injury, however did not find statistically significant difference between two and four weeks, indicating that the nerve damage is permanent and the two week interval between the nerve injury and erectile function evaluation is sufficient.

The parameters for electrical stimulation have been previously studied and optimal stimulation parameters have been established (18). One area of variability, however, is the length of rest period between stimulations. Most studies use a 5 to 10 minute rest period between stimulations (19-21). This makes the experiment long and necessitates re-injection of anesthetic, which in turn affects the erectile response and confounds results. Here we demonstrate that the rest interval could be shortened to 1 minute in the context of a stable nerve-electrode complex that does not need to be manipulated/dried between stimulations. Additionally, different types of anesthesia have different effects on hemodynamics and neural function. Depth and type of anesthesia clearly affect erectile function. According to the literature search and our own observation we believe that the ideal anesthesia is ketamine/kylazine or fentanyl, fluanisone, midazolam. We have also shown that adding oxygen through a nose cone increases the ICP. This is significant because none of the published studies mention use of oxygen (18).

This study is not without limitations. The cavernous nerve is not the only nerve involved in erectile function. Up to 50% of the rat penis is innervated from the ancillary nerves (22). These nerves are not affected by bilateral CM nerve crush injury. The unaffected ancillary nerves can explain why the ICP is not zero in our observations. Therefore, our observed reduction in erectile function cannot directly be correlated to the grade of ED that develops in the men after radical prostatectomy. Additionally, this model continues to be a technically challenging procedure that may vary based on operator.

Legends

### **Figure 1**

**Study groups.** Twenty-four Sprague-Dawley rats were divided into three study groups: Intracavernous pressure (ICP) measurement was performed in control healthy animals and in animals 2 weeks and 4 weeks post cavernous nerve (CN) crush injury.

### **Figure 2**

**CN dissection.** The major pelvic ganglion (MPG) and cavernous nerve (CN) running on the dorsolateral aspect of the prostate. (Reproduced with permission from Ref. 18)

### **Figure 3**

**Exposing the penile crus.** (A) Vertical skin incision starting 0.5 cm lateral to the base of the penis. (B) The ischiocavernosus muscle (arrow). (C) The tunica albuginea appears bright white (arrow). (Reproduced with permission from Ref. 18)

### **Figure 4**

**Placement of the line for penile cannulation.** The line introduced through the small skin incision, running parallel with cavernous body and connected to a needle (arrow). (Reproduced with permission from Ref. 18)

### **Figure 5**

**Isolation of CN.** (A) Freeing the cavernous nerve (CN) from the prostate and isolation from the overlying fascia. (B) Silver hook electrode with tips, stripped from Teflon, placed underneath the CN. (Reproduced with permission from Ref. 18)

### **Figure 6**

**Application of silicone glue.** The nerve-electrode complex is lifted using micromanipulator and held while the two-component silicone glue is applied and for additional 2 – 3 minutes until the glue dries. After, the tension on the electrode is released. (Reproduced with permission from Ref. 18)

### **Figure 7**

**Cannulation of the penile crus.** The needle is introduced into the penile crus parallel to its course.

**Figure 8**

**Maximum and mean intracavernous pressures.** In the control group, the maximum intracavernous pressure (ICP) was  $82 \pm 4$  and the mean ICP was  $71 \pm 5$ . In the two week post-injury group, the maximum ICP was  $53 \pm 4$  mmHg and the mean ICP was  $41 \pm 4$  mmHg. In the four week post-injury group, the maximum ICP was  $57 \pm 3$  mmHg and the mean ICP was  $39 \pm 2$  mmHg.

**Figure 9**

**Area under the curve.** The area under the curve was  $39.4 \pm 2.3$  in the control group and  $22.7 \pm 1.5$  and  $21.9 \pm 1.2$  in the animals two and four weeks post cavernous nerve crush injury, respectively.

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## Figures

Figure 1

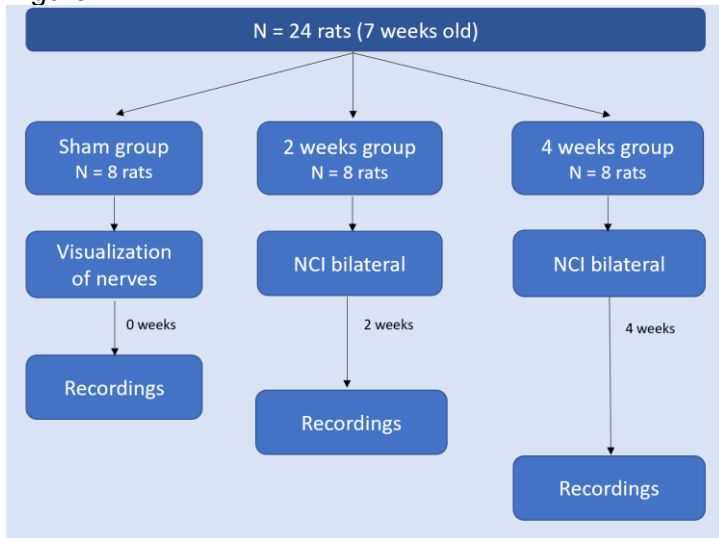


Figure 2

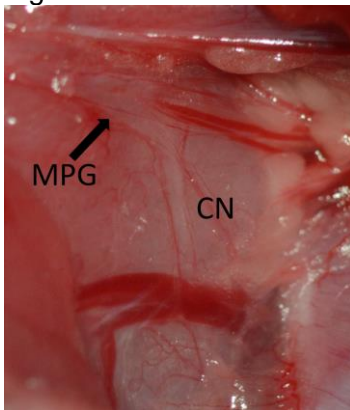


Figure 3

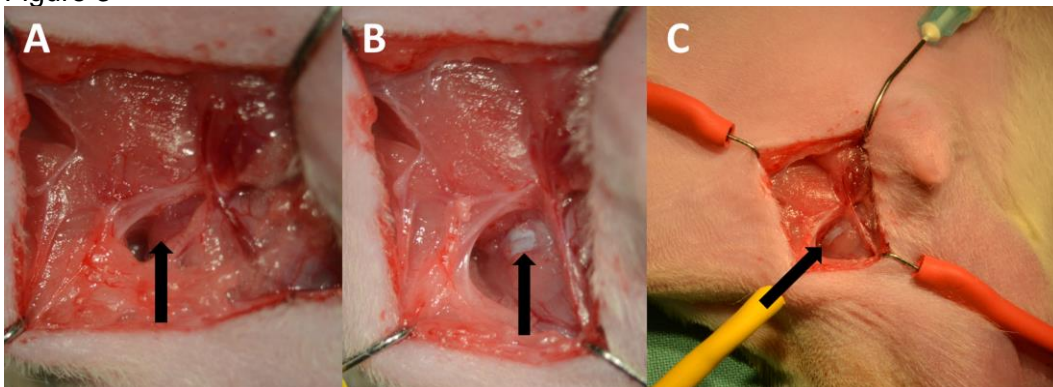




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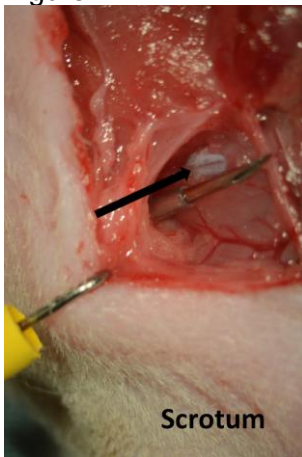


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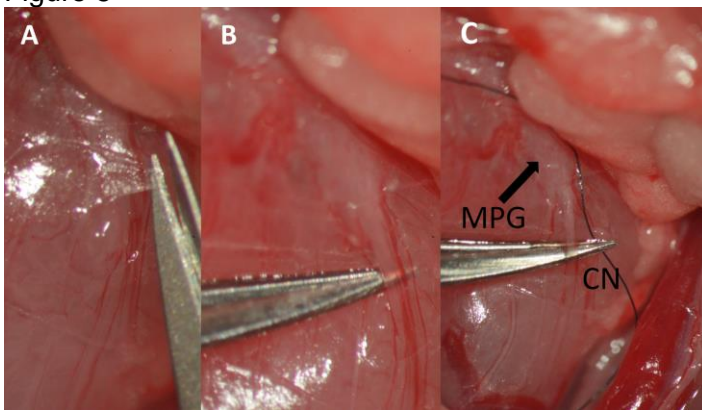


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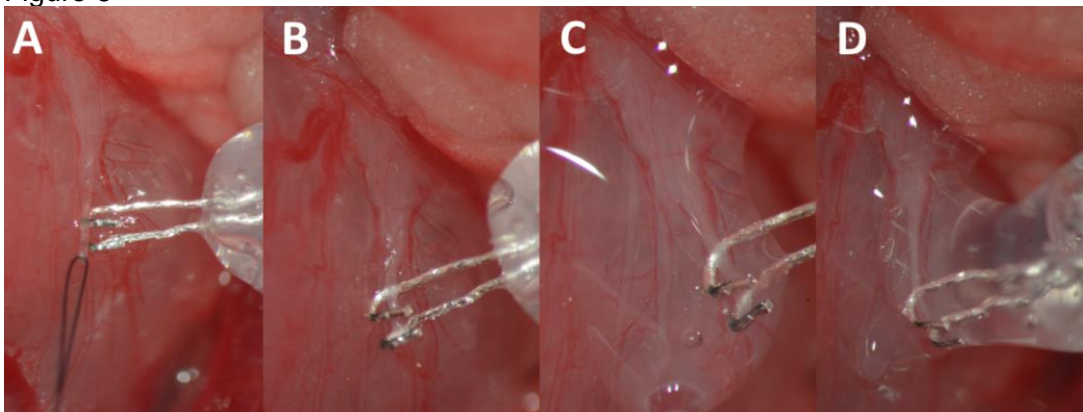


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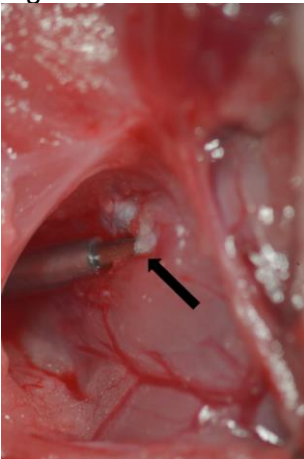


Figure 8

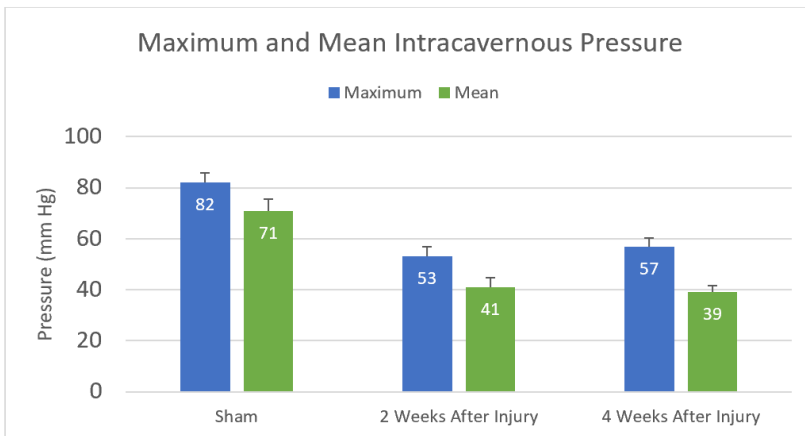


Figure 9

