# European Society for Sexual Medicine Consensus Statement on the Use of the Cavernous Nerve Injury Rodent Model to Study Postradical Prostatectomy Erectile Dysfunction

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## ABSTRACT

**Introduction:** Rodent animal models are currently the most used in vivo model in translational studies looking into the pathophysiology of erectile dysfunction after nerve-sparing radical prostatectomy.

**Aim:** This European Society for Sexual Medicine (ESSM) statement aims to guide scientists toward utilization of the rodent model in an appropriate, timely, and proficient fashion.

**Methods:** MEDLINE and EMBASE databases were searched for basic science studies, using a rodent animal model, looking into the consequence of pelvic nerve injury on erectile function.

**Main outcome measures:** The authors present a consensus on how to best perform experiments with this rodent model, the details of the technique, and highlight possible pitfalls.

**Results:** Owing to the specific issue—basic science—Oxford 2011 Levels of Evidence criteria cannot be applied. However, ESSM statements on this topic will be provided in which we summarize the ESSM position on various aspects of the model such as the use of the Animal Research Reporting In Vivo Experiments guideline and the of common range parameter for nerve stimulation. We also highlighted the translational limits of the model.

**Conclusion:** The following statements were formulated as a suggestive guidance for scientists using the cavernous nerve injury model. With this, we hope to standardize and further improve the quality of research in this field. It must be noted that this model has its limitations. **Weyne E, Ilg MM, Cakir OO, et al. European Society for Sexual Medicine Consensus Statement on the Use of the Cavernous Nerve Injury Rodent Model to Study Postradical Prostatectomy Erectile Dysfunction. Sex Med 2020;8:327–337.**



**Key Words:** Animal Model; Erectile Dysfunction; Radical Prostatectomy; Cavernous Nerve

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## INTRODUCTION

The advent of prostate specific antigen screening has led to the early detection of prostate cancer (PCa). As a conclusion, more and more patients will have good long-term oncologic outcome after radical prostatectomy (RP).[1–3] In contrast, it has been reported that only 23% of men younger than 60 years regain their complete erectile function (EF) after bilateral nerve-sparing RP (NSRP).[4]

In accordance with the most recent literature,[5] patient preoperative and intraoperative factors (including age, preoperative EF, comorbidities), type of surgery (unilateral vs bilateral nerve-sparing), grade of nerve sparing (ie, intrafascial vs interfascial vs extrafascial surgeries), and surgical skill represent the key significant contributors to EF recovery after NSRP.[5]

In nerve-sparing surgery, erectile dysfunction (ED) occurs as a consequence of iatrogenic damage to the periprostatic neurovascular bundle, which results in neuropraxia of the cavernous nerves (CNs).[5–9] Neurapraxia, as per the Seddon classification, is a temporary failure of nerve conduction because of a damage to the myelin sheath.[10,11] The Seddon classification of nerve injury separates injury based on the scale from microscopic to macroscopic along with severity of tissue injury, prognosis, and time for recovery. Seddon described 3 types of nerve injury—neurapraxia, axonotmesis, and neurotmesis[12] with each type having a different potential for regeneration. Neurapraxia, the temporary block of the CN conduction, results in a decrease of the rate and quality of both daily and nocturnal erections, and it promotes a persistent cavernous hypoxia.[10,11] In vitro and in vivo studies suggested that the neurapraxia and the consequent penile hypoxia might result in collagen accumulation, smooth-muscle apoptosis, and fibrosis.[13,14]

Finally, these penile changes lead to venous leakage and permanent ED before complete recovery of the nerve integrity can be achieved up to 2 years after surgery.[15] It has been shown that ED occurring after RP differs from classical vasculogenic ED.[16–19] In human corpus cavernosum tissue, endothelial function was preserved in patients after RP, whereas significant disturbances were seen in neurogenic relaxation with sympathetic hyper innervation. Accordingly, molecular analysis of protein expression showed significant changes of neuronal proteins in post-RP ED which differs from that observed in vasculogenic ED.[16] In line to what is observed in humans, it was reported that penile endothelial function was preserved in rats after crushing of the CNs.[18,19] In contrast, imbalanced neurogenic responses favoring adrenergic contraction over nitrergic relaxations have been observed in the human tissue and rat model.[17,19] This concept is very important because recovery of endothelial function has been used as an end point in many interventional studies in rats investigating therapeutics in post-RP ED.[20]

Animal models mimicking this CN injury (CNI) have played a role in the advancement of the field.[21,22] Rodent models have become the standard in early phases of in vivo research method because of their relatively low cost compared with other animal species.[21–23] Both mouse and rat animal models are available, with mice potentially allowing higher throughput in some cases, while also providing options for genetic knockout or modification.[24] However, despite the possibility of genetic engineering providing a valuable tool for investigation, evaluation of EF may be more challenging in the mouse than in the rat model.[25]Various preclinical studies, using NSRP rodent models, have demonstrated that vacuum erection devices and several medications (ie, alprostadil injections, phosphodiesterase type 5 inhibitors, and so on) are able to promote EF recovery, improve the cavernosal smooth-muscle/collagen ratio, increase penile smooth-muscle replication, reduce penile apoptosis, preserve penile endothelial function, increase antioxidant enzymes, and promote neuroprotection during and after neuropraxia.[22]

In spite of promising results generated in the rodent models, most of the well-designed clinical trials have failed to confirm any structural or lasting benefit of this type of treatment in improving the recovery of EF after RP.[5,7,8,26–28]

One of the reasons for the discrepancy between the clinical and animal data may be because of variance in the methodology used when conducting the basic science studies.[22] The variance in the methodology and the lack of consensus guidelines for the use of the NSRP rat model (also called bilateral CN crush, transection, excision, dissection, freezing, electrocautery, and irradiation model) have led to the publication of studies whose results are often equivocal and impossible to compare.[29]

The aim of this review is to review the current state of art, highlight possible pitfalls, and provide statements for experimental technique and reporting in the use of the NSRP rat model; to provide further standardization and improvement in the quality of research in this field on behalf of the European Society of Sexual Medicine (ESSM).

## METHODS

MEDLINE and EMBASE databases were searched for articles looking with the following search terms: ("erectile dysfunction" OR "erectile function" OR "radical prostatectomy" OR "post-RP ED" OR "cavernous nerve injury") AND ("animal model" OR "model"). Studies from 1980 up to 2019 were included.

Abstracts were screened for relevance (F.C. and E.W.); if it was not clear from the abstract whether the article may contain relevant data, the full article was assessed.

Only the basic science studies, using a rodent animal model, looking into the consequence of pelvic nerve injury on EF were considered relevant for this study. Thereafter, relevant studies were analyzed and summarized after an interactive peer-review process of the panel (F.C., E.W., D.B.R., M.I., A.M., and C.G.) to obtain a narrative review. The statements were internally discussed. Disagreements were resolved by consensus. The study was internally reviewed by senior authors (M.A., C.B., Y.C., J.A.).

Owing to the specific issue—basic science—Oxford 2011 Levels of Evidence criteria cannot be applied. However, ESSM statements on this topic will be provided in which we summarize the ESSM position on various aspects of the model.

Ethical board approval was not required for this work.

## GENERAL CONCEPTS OF TRANSLATIONAL RESEARCH—THE ANIMAL RESEARCH REPORTING IN VIVO EXPERIMENTS GUIDELINE

Statement #1: The ARRIVE check list should be completed and submitted with the manuscript. In particular, the strain, sex, weight, and age of animals always needs to be reported.

### Evidence

The initial basis for addressing a clinical problem in an experimental setting is the selection of a valid model that corresponds to the human condition in etiology, pathophysiology, symptomatology, and response to therapeutic interventions.

The key to using any method or model is to understand its limits and what outcomes it measures, represents, and is able to forecast. For animal models, comparable biological processes or behaviors to signs, responses, or symptoms of human functions and disease, that is face validity, are important for translational research.[30] In sexual arousal, penile erection is an example of a component of male sexual behavior that in several species share substantial physiological and biochemical events and for which methods exist that can be used to assess similar end points.[31–35] Human clinical trials are regulated by authorities to reduce biases by patients and investigators, whereas corresponding controls do not seem to be extensively in effect in medical research with animals, that despite more objective end points, still is at risk for unconscious procedural errors.[36] To increase the value of research processes with animals, we emphasize the concept that the ARRIVE (Animal Research Reporting In Vivo Experiments) guidelines and recommendations should be followed.[25] In particular, the ARRIVE guidelines were developed to improve standards of reporting and ensure that the data from animal experiments can be fully evaluated and used. They consist of a 20-point checklist (https://www.nc3rs.org.uk/sites/default/files/documents/Guidelines/NC3Rs%20ARRIVE%20Guidelines%20Checklist%20(fillable).pdf) of the essential information that should be included in publications reporting animal research. No study has so far examined how authors of publications involving CNI in rodent models adhered to the ARRIVE guidelines. However, there is a recent survey of 271 publications between 1999 and 2005, which involved the use of live rats, mice, and non-human primates, carried out in the UK and US publicly funded research establishments. This survey provided evidence that many peer-reviewed, animal research publications fail to report important information regarding animal demographics and experimental protocols.[37] To mention some examples from this survey, 24% of studies did neither report the age nor the weight of animals, 12% of studies reported random allocation of animals to groups, 5.9% used investigator blinding to treatment, and in 4% of the studies, the statistical method was unclear.[37]

### Remarks

Similar potential deficiencies in animal research are noted in several medical areas, including sexual medicine, and correlations have been made between insufficient reporting and translational value.[36] Few articles in the sexual medicine field have reported the use of the ARRIVE checklist.[38–41]This concept is very important because similar to humans, several mammals display —in aging or because of various diseases—signs of ED and alterations of important regulatory pathways of erection.[24] The lack of crucial information can prevent the correct evaluation of the final results obtained. The ARRIVE checklist should be completed and submitted with the manuscript involving animal models for radical retropubic prostatectomy.

## PHYSIOLOGICAL INVESTIGATIONS OF PENILE ERECTION—THE RAT ANIMAL MODEL

Extensive research suggests that regulatory mechanisms of penile erection exhibit substantial physiological, biochemical, and pharmacologic homologies among mammalian species, including humans.[31–33] For example, human, monkey, rabbit, dog, rat, and mouse corpus cavernosum tissue that is precontracted to simulate penile flaccidity responds similarly via neuronal and endothelial signals with relevant nitric oxide (NO)-dependent relaxant "erection-simulating" responses.[42–47] Accordingly, a crucial role for the nitric oxide pathway in penile erection has been characterized in vivo during erections in various animals.[38,48–52] However, peculiar species characteristics are also present. Hence, the selection of the correct animal model and the parameters to be tested is essential in transitional medicine (see the following sections).

### Measurement of Intracavernous and Corpus Spongiosum Pressure

Statement #2: The use of intracavernous pressure (ICP) registrations should be preferred to corpus spongiosum pressure (CSP) as an end point during erection.

Statement #3: The Methods section should clearly report if ICP or CSP was recorded.

### Evidence

The human corpus spongiosum is responsible for engorgement of the glans during the erection. The human bulbospongiosus muscle that surrounded the corpus spongiosum has an important role to propel semen during the expulsion phase of ejaculation.[53–56] In rats that have a peculiar anatomy of the penis with an almost 180° frontal flex of the glans, penile striated muscle activity is also essential to compress the erectile compartment to straighten the glans penis ("flips") to achieve

intromission.[57] After transection of the CN, the rat can still achieve erections of the glans.[57] Furthermore, in contrast to humans, rats also use the bulbospongiosus muscle together with a well-developed rhabdosphincter to forcefully and rhythmically expel urine during micturition.[58–60] Consequently, significant pressure responses are recorded during rat micturition, and therefore, for the use of CSP registration in awake and freely moving rats, the behavioral context may need particular attention.[60]

### Remarks

Some investigators have studied CSP as an end point during erection.[61–63] When compared with the corpora cavernosa, the corpus spongiosum has a less-firm tunica albuginea and arteriovenous shunts in the glans and may be considered to be a flow-through compartment with lower pressures during erection.[64,65] Owing to this potential bias, the use of ICP registrations should be preferred to CSP as an endpoint during erection.

## ICP and Mean Arterial Pressure

Statement #4: ICP should be normalized by mean arterial blood pressure (MAP); especially when vasoactive substances are used.

Statement #5: Detailed description of the method used to record ICP and MAP should be reported.

Statement #6: Exemplary and representative images of the ICP and MAP traces should be included in or attached to the manuscript.

### Evidence

Activation of the pelvic nerve or the cavernous (penile) nerve by contact electrodes has been shown to induce analogous penile erections in monkeys, dogs, rabbits, rats, and mice that are recorded as characteristic changes in ICP.[66–69] Correspondingly, in patients undergoing NSRP or penile surgery, stimulation of the neurovascular bundle posterolateral to the prostate induced subjectively assessed erections or penile tumescence, and activation of the CNs similarly caused visible erections that were correlated to simultaneous increases in ICP.[65,70] Hence, under these investigative conditions, techniques to study nerve-induced increases in ICP in animals seem to comprise appropriate translational models for humans.

ICP measurement in rodent can be achieved by implanting the tip of a recording catheter into the corpus cavernosum and connecting to a pressure transducer.[62]

To register ICP responses, commonly either the crura or the body of the corpus cavernosum is cannulated using either PE tubes or needles. However, few studies exist that compare the cannulation sites.[71] The insertion of the catheter can be performed using a small needle connected to the end of the recording catheter or via an incision of the tunica albuginea of the penis, fixing it with a purse-string suture. Before and after the insertion the catheter and the needle, if used, should be flushed with a solution containing heparin to avoid clots. There are no studies that have investigated differences in the outline of the experimental setup. To register systemic blood pressure, a large artery, commonly the aorta or the carotid artery, is cannulated using either PE tubes or needles filled with heparin.[72]

### Remarks

Because arterial blood pressure may affect ICP responses, a standard experimental setup should include procedures for simultaneous recording of pressures from the erectile compartment and central arteries.[73] Consequently, the amplitude and/or area under curve of the erectile responses after electrical stimulation of the CN should be normalized by the MAP during the erectile response and reported as such.[64] As ICP can be affected by hemodynamics, it is highly recommended that the ICP is adjusted by the MAP and reported as ICP/MAP especially when vasoactive substance are used.[73] Exemplary traces of ICP and MAP are usually included in the manuscript as to provide the reader and reviewers with the option of assessing quality of registration/stimulation.[74]

The authors should clearly report and describe the measures recorded during the experiment. These should include how many electrical stimulations were performed for each animal, the length of electrical stimulation, and if ICP mean or peak is used in the calculation. The authors should also clearly report the methodology and the instruments that were used during the experiment including: the site of (crura or corpora) the catheter insertion, the size and the material of the catheters used, the size of the needle used, the amount and the concentration of heparin that was administrated, and the instrument used for the crush and for how long the crush was performed. The version of recording machine and software used should be accurately described.

## Type of Rodent Models

Statement #7: Rodent models should be used to mimic post-RP ED. While mouse models might offer higher throughput and options for genetic modification, rat models might offer more ease of use for CN stimulation.

### Evidence

The rat is the most commonly used species for in vivo studies of erection. Electrical stimulation of the CN can cause reproducible ICP responses after appropriate training.[29,33,75] The rat bilaterally has a single CN that is readily identified running from the pelvic ganglion on the lateral side of the prostate (Figure 1), whereas corresponding structures in humans are derived from a more diffuse nervous meshwork of the neurovascular bundle.[75–77] Behavioral, neurophysiological, and molecular biological or genetic procedures are conveniently available for multimodal investigations when using rats, and animal purchase, housing, and maintenance costs are relatively low. While mice

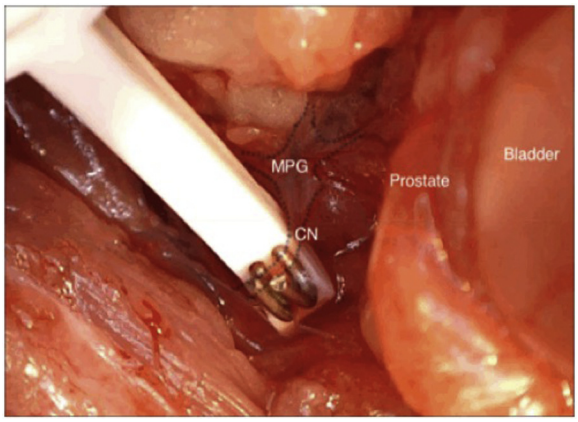


**Figure 1.** Rat cavernous nerve stimulation. CN = cavernous nerve; MPG = major pelvic ganglion.

might offer a higher experimental throughput in some cases, the CN stimulation as well as the ICP recording might be easily achieved in the rat model.[33,78] It should be noted that the rat is reported to exhibit ancillary penile innervation from the major pelvic ganglion, which accounts for around 45% of ICP responses to supraspinal stimuli after CN transection.[76] These ancillary nerves are not damaged in the rat model of RP.

### Remark

The lack of damage to these ancillary nerves represents an element to be considered in the design of the experiment representing a possible source of bias. However, there are no studies that have tested the long-term effect of the ancillary nerves on the EF of the rat.

## Parameters for CN Electrical Stimulation

Statement #8: Detailed description of the parameters for CN electrical stimulation, including: pulse duration, frequency, duration of stimulation, voltage and rest period, should be reported.

Statement #9: The most commonly agreed parameter ranges for cavernous nerve stimulation in rats are pulse duration, 0.5 ms−1 ms, frequency, 10−20 Hz; duration of stimulation, 30−60 seconds; voltage, 2.5−8V (L4). Frequency- or voltage-response curves should be established to manifest optimal and suboptimal stimulation parameters. Rest periods of at least 5−10 minutes between subsequent stimulations should be applied. We recommend using these parameters to improve comparability between studies.

### Evidence

Most researchers have reported the stimulus in volts and values ranged from 1 to 15V.[75,79−81,71,82,83] Other groups have reported the intensity of the current (ampere) for characterizing the procedure of nerve activation, and this parameter ranged between 0.5−10 mA.[71,84−86] The frequencies (Hertz) of the stimuli used by the aforementioned research groups varied between 1 and 30 Hz. Optimal (maximal) ICP responses are generally reported around 6−7.5 V or 1.5 mA and between 10 and 20 Hz.[79,71,84,87] Ideally, a voltage-response or intensity-response or frequency-response curve is produced to depict erectile responses elicited at several electrical stimulation parameters as this may yield interesting information on the erectile effect a compound/device may produce (at low-, intermediate-, or high-stimulation parameters). Another parameter of interest for nerve stimulation is the width (or duration) of the single pulse to differentiate the threshold for activation of different types of nerves.[88] This parameter is reported to vary from 0.05 to 5 ms.[80,81,71,84−87,89] Considering settings for optimal stimulation of the vagus nerve in rats, CN activation in dogs, or these used for intraoperative activation of the CN in humans, a pulse width around 0.2 ms is probably appropriate for rodent ICP models.[65,90−93] In the mouse ICP model, 0.5−6V, 5−20 Hz, and pulse durations of 1−5 ms have been used to activate the CN.[66,94] Maximal ICP responses were reported at 3V and 15 Hz.[66]

### Remarks

A quite high variability of electrical stimulation parameters used by different research groups has been reported. This may in part be related to the type of stimulators used whether the voltage or the current can be regulated. For voltage stimulators, the resistance of the electrode may affect the intensity of the current needed to activate the nerve. Conversely, stimulators that adjust for the resistance in the electrode delivers stable currents for nerve activation.[65] We recommend using the most commonly used parameters (outlined previously) to improve comparability between studies.

## Site for CN Electrical Stimulation

Statement #10: The author should clearly report if a unilateral or bilateral CN stimulation were performed.

Statement #11: The author should clearly report the location of nerve stimulation that is proximally or distal to the point of the nerve damage.

### Evidence

Recording erections with a video camera, Quinlan et al [75] observed better responses upon bilateral stimulation of the CN as compared with unilateral nerve activation. Even so, most of the following studies have implemented unilateral stimulation of the CN, and it may be discussed if the neurovascular mechanisms responsible for ICP responses are fully activated under such conditions, hence the advantage of performing a response curve to several electrical stimulation parameters to reach maximal response. As reported by registration of ICP in dogs, unilateral stimulation of the CN induced full erection of both the corpora cavernosa even if the ICP responses were achieved faster by bilateral stimulation.[91]

### Remarks

Unilateral CN stimulation may give false low-filling values for tumescence and possibly display a delayed onset of the veno-

**Table 1.** Methods of rat nerve injury

| Method of nerve injury | Comment | Details | Reference |
|---|---|---|---|
| Crush CN injury | Resembling nerve sparing RP | Crushing CN by applying instrument distal of MPG (eg, forceps 3 × 15 seconds or hemostat 3 minutes). | 5 |
| Excision CN injury | Resembling non-nerve sparing RP | Segment of CN is removed, resulting in a gap between nerve endings. | 92 |
| Transection CN injury | Resembling non-nerve sparing RP | Division of CN, nerve endings remain close by. | 95 |
| Unilateral nerve injury | Nerve sprouting and maintenance of erections from contralateral side are disadvantages | 1 CN injured, other unharmed. | 70 |
| Bilateral nerve injury | Considered to be the gold standard and most reliable to study erectile function recovery | Both CN are injured. | 70 |
| Stretching injury | Difficult to standardize | Nerve is dissected and held under traction. | 96 |
| Freezing injury | Unnatural method of injury | CN is frozen with contact to dry ice or thermocoupling probe. | 32 |
| Dissecting injury | Difficult to standardize | CN is dissected around the prostate and isolated without deliberately injuring. | 33 |

CN = cavernous nerve; MPG = major pelvic ganglion; RP = radical prostatectomy.

occlusive mechanisms. It must be noted, however, that most of the studies currently performed in rats use unilateral nerve stimulation. The unilateral CNI model can serve as its own control; the injured and sham groups are in the same animal at the same time, while having the disadvantage of potential compensatory action of the intact nerve. More importantly, the location of nerve stimulation that is proximally or distal to the point of nerve damage nerve damage could influence the ICP response. An electrical stimulation proximal to the point of nerve damage evaluates both the component of nerve damage and those of damage to erectile tissue. Distal nerve damage stimulation evaluates only the component of erectile tissue by bypassing nerve damage.

## Type of CNI

Statement #12: Bilateral and not unilateral CNI should be considered as the standard animal model for human RP.

Statement #13: Detailed description of the parameters for CNI including mode of injury (crush, transection, cold, heat,), type of instrument used, and duration of injury and should be reported for the purpose of reproducibility of the model.

### Evidence

Both unilateral and bilateral CNI models have been used to study ED and are believed to imitate the condition in humans after RP. Various types of CNI have been studied in rodent models, especially rats.[78] These models were stratified by the type and extent of injury. Various types of injury techniques such as stretching, crushing, freezing, transecting, dissecting, and excising the CN as well as unilateral vs bilateral CNI have been described[22] (Table 1). Crushing of CNs is most commonly used to mimic the nerve injury that occurs when using the NSRP technique.[22] On the other hand, transection and excision of the CNs are mostly used to mimic RP without nerve-sparing procedure.[29] The crush injury model involves various mechanical compressions of the CN over varying periods of compression time.[6,26,72,95] The crushing can be induced by several instruments (forceps, hemostatic, or bulldog clamps, microserrefine serrated or not).It is not clear if the instrument used or the timing of the crush can induce any difference in the magnitude and consistency of provoked ED.[71] Transection injury consists of direct division of the CN, whereas excision CNI implicates the removal of a segment of CN.[96] CNI can be applied unilaterally or bilaterally.

### Remarks

In unilateral nerve injury models, mimicking unilateral NSRP, the nerve supply is partly preserved and therefore penile elections partly maintained.[76] Furthermore, compensatory nerve sprouting from the intact contralateral CN is believed to occur.[10] In unilateral injury models, the contralateral side is often used as a control, but as mentioned, contralateral sprouting can confound experimental interpretation.[10] Because of these reasons, bilateral and not unilateral CNI is considered as the standard model.[22,78]

## Limitations

Statement #14: Rodent models for erectile dysfunction after NSRP used in experimental studies present important limitations when compared with the clinical settings. These limitations should be reported.

Statement #15: The author should consider a possible source of discrepancy between basic science studies and the clinical practice. The limitations are as follows: the age of the rat involved in the experiment, the absence of rat comorbidities, and the spontaneous recovery of erectile function of the rat after 6 months.

### Evidence

For rat animal studies, male rats between the age of 10 and 12 weeks or weighing between 300 and 400 grams are generally used.[29,22] It has been criticized that the age of these rats corresponds with adolescence in humans and not middle-aged men such as typically seen in patients with PCa .[97] Days or months after the CN injuries, the EF is evaluated as per the Quinlan et al[75] model by the electrical stimulation of the injured nerve.[98] In rodent studies, the exact time frame in which maximal nerve regeneration and EF recovery occurs is not uniformly agreed upon. Commonly, EF is evaluated 4 weeks after nerve injury in rats because it is generally believed to represent the 2-year time in humans.[99] Indeed, in humans, EF continues to improve up to 24 months after RP.[100] As early as 1 day and up to a week after CN crush, only 30−40% of axons survive and maximal ICP response to CN stimulation are 4 times lower than in control animals.[101,102] It has been shown that EF is decreased 48 hours after bilateral crush CNI and starts to recover at 60 days after injury.[103] Spontaneous complete recovery of EF has been seen 6 months after bilateral CN crush injury after injury.[104]

Rodent models have been used to study pathophysiologic mechanisms and assorted pharmacologic, surgical, and regenerative treatments.[22] Several preclinical and translational studies have shown a benefit of penile rehabilitation therapies such as phosphodiesterase type 5 inhibitor/GC activator treatment,[105] immunomodulation, neurotrophic factor administration, and regenerative medicine options such as stem cell therapy in animals.[22] However, most of these approaches have either failed clinical translation or have yet to be studied in human subjects.[11]

### Remarks

The reason of these clinical translation failures may lie in the limits that characterize the CNI model and in the design bias which can occur in basic science studies:

1) The EF evaluation in rats is performed by an objective method (ICP evaluation, electrical stimulation of CN) and, on the other hand, in the clinical practice the evaluation is performed via questionnaires.[11]
2) As mentioned, there are reports that the ancillary nerves provide more than 50% of the proerectile innervation to the rat penis.[76] These ancillary nerves remain unaffected in the CNI model, and it is unclear if this might have an impact on the severity of ED in rats compared with humans. More important, the CNI mimics a "perfect "bilateral intrafacial nerve-sparing procedure difficult to perform in all the patients in the clinical setting.
3) The CNI studies, commonly use 10- to 12-week-old and thus healthy young rats without any baseline ED.[29,22] This does not reflect the current clinical landscape. Patients with PCa are commonly older than 50 years of age and may be suffering from different comorbidities (diabetes, cardiovascular disease) which cause baseline ED and can impair nerve repair mechanisms after the NSRP.[15] This discrepancy between basic science studies and the clinical practice is illustrated in several clinical studies which showed that young patients without comorbidities have a better recovery of EF after NSRP compared with other patients.[5,8,106] Moreover, preoperative EF and Carlson Comorbidity Index are considered independent predictors for EF recovery.[15]
4) The spontaneous recovery of EF in the CNI rat model[104] has important scientific-translational consequences. Indeed, this model can only be used to assess if a treatment can modify the time to return of EF recovery. Conversely, this model cannot be used to test if there is an absolute variation in terms of EF recovery rate between 2 or several treatments. The spontaneous recovery of EF in the CNI rat model should be considered as one of the major limitations of this model.
5) Several studies in this field lack high quality and correct in vitro experimental methodology. Describing the various in vitro techniques in detail is beyond the intent of this ESSM statement; however, we provided more information on the different available in vitro methods (such as common stainings and marker proteins) to assess fibrosis, neural, and endothelial in tissues in the supplementary data (supplementary data -Table 1)

## CONCLUSION

Today, the rat is commonly used as an animal model to mimic post-RP ED by bilaterally crushing the CNs. In this document, we provided ESSM statements for the correct and reproducible use of this CNI model. We hope to increase comparability between reports which could advance the field as a whole. It must be noted that this model has its limitations. This may help to narrow the gap between preclinical studies and their translation in clinical practice.

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## SUPPLEMENTARY DATA

Supplementary data related to this article can be found at https://doi.org/10.1016/j.esxm.2020.06.007.