Video Article

Nerve-sparing Mid-urethral Obstruction (NeMO) in Female Small Rodents

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URL: https://www.jove.com/video/55288
DOI: doi:10.3791/55288

Keywords: Medicine, Issue 122, partial bladder outlet obstruction, mid-urethral obstruction, bladder hypertrophy, bladder remodeling, neurogenic bladder, animal model

Date Published: 4/25/2017

Citation: Sidler, M., Aitken, K.J., Jiang, J.X., Bägli, D.J. Nerve-sparing Mid-urethral Obstruction (NeMO) in Female Small Rodents. *J. Vis. Exp.* (122), e55288, doi:10.3791/55288 (2017).

Abstract

Partial bladder outlet obstruction (pBOO) has a high prevalence, causes significant patient burden, and immense health care costs. The most common animal model to investigate bladder remodeling in pBOO are female rodents undergoing partial obstruction at the proximal urethra. Variability in the degree of obstruction and animal mortality are major concerns with proximal obstruction. Furthermore, dissecting around the proximal urethra and bladder neck jeopardizes bladder innervation.

We developed a nerve-sparing mid-urethral obstruction (NeMO) model for pBOO avoiding the disadvantages of the traditional model. We approached the urethra just inferior to the pubic symphysis, which obviated the need for laparotomy as well as for dissection in this area; also, the striated urethral sphincter remained untouched. We performed NeMO in female Sprague-Dawley rats (12 obstructions, 6 sham animals) as well as in female C57/bl6 mice (20 obstructions, 18 sham animals). After two weeks, we evaluated bladder function, bladder mass, and body mass.

We had no mortalities among obstructed- or sham-operated female rats; as described for the traditional proximal pBOO-method, we tied the suture around the proximal urethra and a temporarily placed 0.9 mm metal rod. NeMO induced an 85% increase in bladder mass after two weeks, average residual urine volume was 0.4 mL in partially obstructed rats while only 0.03 mL in sham animals. In mice, we tested 3 sizes of cannulas that we placed along the urethra when tying the suture. We found that using a 27-gauge cannula resulted in over 50% animal mortality; placing the 25-gauge cannula did not yield the desired response in increasing bladder mass; utilizing a 26-gauge cannula yielded favorable results with minimal animal mortality (1/8) yet a significant 2-fold increase in bladder mass.

Video Link

The video component of this article can be found at https://www.jove.com/video/55288/

Introduction

Partial bladder outlet obstruction (pBOO) has a high prevalence and can result in severe bladder dysfunction[1]; the spectrum ranges from congenital malformations such as posterior urethral valves or hypospadias, over acquired urethral strictures to benign prostatic hyperplasia. The latter affecting more than 30% of men sixty years and older[2]. Great patient burden and immense health care costs associated with pBOO warrant the considerable research effort put into studying bladder remodeling in response to increased outflow resistance[3]. From 2006 until 2015, over 220 PubMed indexed articles were published concerning the effect of pBOO on the urinary bladder.

Although animal models for pBOO have been devised in several species[4], such as the rat[5], the rabbit[6], pig[7], and mouse[8,9], arguably the most commonly used animal model however are female rats undergoing partial obstruction of the proximal urethra; access to the animals' abdomen, exteriorizing of the bladder, and dissection around the bladder neck are inevitable with this traditional proximal urethral obstruction technique. Variability in the degree of obstruction and animal mortality are only some of the concerns associated with this procedure[10,11]; we showed that in sham operated animals, dissection around the proximal urethra leads to physiologic changes that correlate with loss of nerve fibers at the bladder neck[12]. This finding indicates, that the most commonly used pBOO animal model, which involves accessing the proximal urethra in female rodents, leads to a denervation injury with associated structural and functional changes of the bladder, affecting sham and obstructed animals. Therefore, an alternate approach avoiding denervation injury was needed. Our lab developed and evaluated a Nerve-sparing Mid-urethral Obstruction (NeMO) approach, effective in inducing expected obstruction-associated changes in the bladder such as increase in organ mass and residual urine, whilst sham-operated animals were indistinguishable from unoperated control animals. Also, the striated urethral sphincter remained untouched as it lies proximal to the level of dissection. Furthermore, variability in obstruction-induced increase in bladder mass was significantly lower than in traditional proximal urethral obstruction and animal mortality was zero.

We also successfully applied NeMO in female mice with a less than 10% mortality in obstructed animals, while all pBOO models for mice described to date were associated with a mortality around 50%. Studying bladder remodeling in the context of pBOO in mice will benefit from applicability of the whole spectrum of transgenic modifications.

Dissecting around the mid-urethra in female rodents does not induce the undesirable and confounding structural or functional changes in the urinary bladder observed in the traditional proximal obstruction model. Nevertheless, inducing a partial obstruction at the mid-urethral level still induces bladder hypertrophy and increased residual urine, as expected from an animal model for pBOO. Importantly, performing NeMO in mice opens investigation of bladder remodeling in pBOO to transgenic methods, which are virtually unavailable in larger rodents.

Protocol

The following experimental protocol was approved by the institution's animal care committee.

1. NeMO in Female Rats

1. **Preparation**
    1. Place a female Sprague-Dawley rat weighing around 200 g under 3% isoflurane anesthesia, weigh the animal, and shave and disinfect the prepubic area 3 times using an iodine surgical scrub.
    2. On a heating pad, tape hind limbs and tail down and catheterize the bladder with a 20-gauge angiocatheter, which is also gently taped down to prevent sliding.
2. **Dissection**
    1. Use a scalpel to perform an 8 mm longitudinal skin incision from the palpable pubic symphysis towards the urethral meatus. Lift off the skin on either side to facilitate later wound closure.
    2. Dissect bluntly to identify the stented urethra by longitudinal spreading with fine scissors. Place fine self-retaining skin hooks to enhance exposure of the urethra.
    3. Perform some gentle longitudinal spreading using curved scissors dorsal to the urethra to develop the plane between urethra and vagina. Then, bluntly pass a 4-0 silk suture behind the urethra and prepare the double throw of a surgeon's knot.
3. **Partial urethral obstruction**
    1. Remove the angiocatheter, place a 0.9 mm metal rod parallel to the urethra, and then tighten the knot around urethra and metal rod, so that the latter still slides out easily thereafter.
    2. Secure the knot with 3 more throws using gentle tension before removing the rod. Cut the suture ends about 3 mm long, or 4 mm if later release of obstruction is part of the experiment.
4. **Wound closure**
    1. Approximate the paraurethral glands using a buried single stitch of absorbable braided suture 4-0.
    2. Close the skin with a buried horizontal mattress stitch using the same absorbable suture. Note: While it may seem unconventional to use an absorbable suture for a skin-penetrating stitch, we did not encounter complications such as wound infections, and that suture removal can be avoided.
5. **After care**
    1. Inject buprenorphine 0.1 mg/kg subcutaneously before placing the rat in a recovery cage.
    2. Offer some soft recovery diet for the first 24 h to the single housed rat. After this period, house animals again in the same pairs as before the procedure provided they are doing well and belong to the same treatment group.
    3. Check the rats daily for bladder size and general well-being; record their weight at least weekly.

2. NeMO in Female Mice

1. **Preparation**
    1. Place a female C57bl/6 mouse weighing around 18 g and proceed as detailed in step 1.1.1.
    2. Proceed as detailed in step 1.1.2, except use a 24-gauge angiocatheter.
2. **Dissection**
    1. Use a scalpel to perform a 6 mm longitudinal skin incision from the palpable pubic symphysis towards the urethral meatus.
    2. Dissect bluntly to identify the stented urethra by longitudinal spreading with fine scissors. Lift off the neuro-vascular bundle running ventrally on the urethra by analogous blunt dissection.
    3. Perform some gentle longitudinal spreading using curved scissors dorsal to the urethra to develop the plane between urethra and vagina. Then, bluntly pass a 5-0 non-absorbable braided suture behind the urethra and prepare the double throw of a surgeon's knot.
3. **Partial urethral obstruction**
    1. Connect the angiocatheter with a 1 mL syringe and inject 0.1 mL of normal saline into the bladder.
    2. Place a 26-gauge cannula parallel to the urethra. Gently tighten the knot around urethra and cannula while pulling back the angiocath.
    3. Secure the knot with 3 more throws using gentle tension before removing the cannula.
    4. Test for urine appearing at the meatus upon gentle pressure on the animal's bladder; loosen the tie if unable to express urine. Otherwise, cut the suture ends about 3 mm long.
4. **Wound closure**

1. Close the skin with a buried horizontal mattress stitch using a 5-0 braided absorbable suture.

5. **After care**
   1. Inject 0.1 mg/kg buprenorphine subcutaneously before placing the mouse in a recovery cage.
   2. Offer some soft recovery diet for the first 48 h to the single housed mouse. After this period, house animals again in the same groups as before the procedure provided they are doing well and belong to the same treatment group.
      NOTE: Mice with a palpable distended bladder 24 h after the procedure that do not void when handled are over-obstructed and will die within the next 1 - 2 days unless taken back under anesthesia for removal of the obstructing ligature.
   3. Check the mice daily for bladder size and general well-being; record their weight at least weekly.

Representative Results

Nerve-sparing Mid-urethral Obstruction (NeMO) in Female Rats

Mortality

We performed a mid-urethral obstruction on over 40 female rats so far and had no mortalities.

Bladder mass

Average relative bladder mass (bladder-to-body-mass-ratio) in sham operated animals was 0.33% two weeks after procedure, while obstructed rats had a mean relative bladder mass of 0.60% which translates into an 85% increase ($p = 0.004$; **Figure 1**).

Residual urine

Sham operated rats had virtually no residual urine that we could aspirate by direct bladder puncture at time of organ harvest, mid-urethral obstruction animals in contrast had a significantly greater mean amount of residual urine of 0.42 mL ($p = 0.01$; **Figure 2**).

Nerve-sparing Mid-urethral Obstruction (NeMO) in Female Mice

Mortality

Overall, 18 female mice underwent a sham NeMO procedure, and one of them died. In order to calibrate the necessary degree of obstruction, we evaluated 3 different diameters of cannulas (25-gauge, 26-gauge, and 27-gauge) placed along the urethra when tying the suture. None of the 5 mice died when we used a 25-gauge cannula as a placeholder along the urethra (**Figure 3**). On the other hand, mortality significantly increased with the smaller size placeholders. 1/8 or 5/8 mice died when using a 26-gauge or 27-gauge cannula, respectively ($p = 0.039$, Fisher's exact test).

Bladder mass

NeMO in female mice using a 25-gauge cannula did not effectively increase bladder to body mass ratio (**Figure 4**). The use of a 26-gauge cannula resulted in an over 2-fold increase in relative bladder mass ($p = 0.04$). When we applied a 27-gauge placeholder an over 60% increase in relative bladder mass occurred after 2 weeks ($p = 0.004$).

Residual urine

Similar to rats, female mice tend to void at induction of anesthesia. When harvesting the bladders, we occluded the bladder neck with forceps and weighed the bladder with urine and after draining the urine, this allowed for precise calculation of residual urine present at bladder harvest. PBOO placing a 25-gauge or a 26-gauge cannula along the urethra did not result in a change in residual urine within 2 weeks (**Figure 5**). Notably, only very few animals voided during induction of anesthesia, which is in contrast to rats that virtually all void when induced. In the 3 mice surviving NeMO using a 27-gauge cannula, mean residual urine volume was increased by 3-fold compared to the respective shams. However, the difference did not achieve statistical significance, most likely due to the low n.

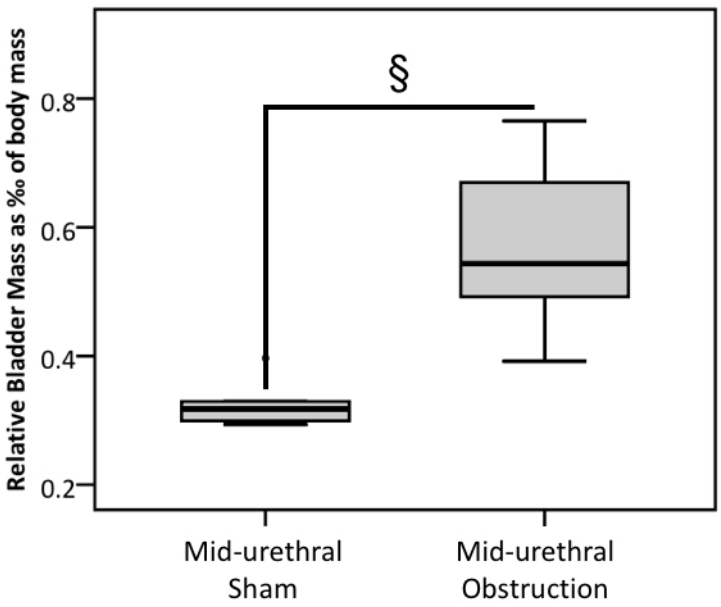

Figure 1. NeMO Effectively Induces Increase in Bladder Mass after 2 Weeks. NeMO led to an 85% increase in bladder-to-body-mass-ratio within 2 weeks (p = 0.004; **§**).

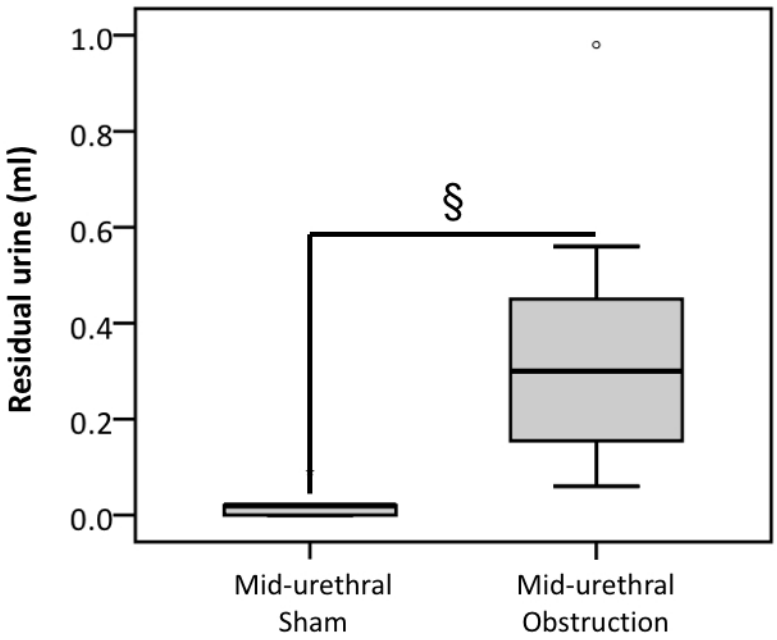

Figure 2. Marked Increase in Residual Urine 2 Weeks after NeMO. While sham operated rats had virtually no residual urine that could be needle-aspirated at time of bladder harvest, mid-urethral obstruction animals had almost 0.4 mL of residual urine at average (p = 0.013; **§**).

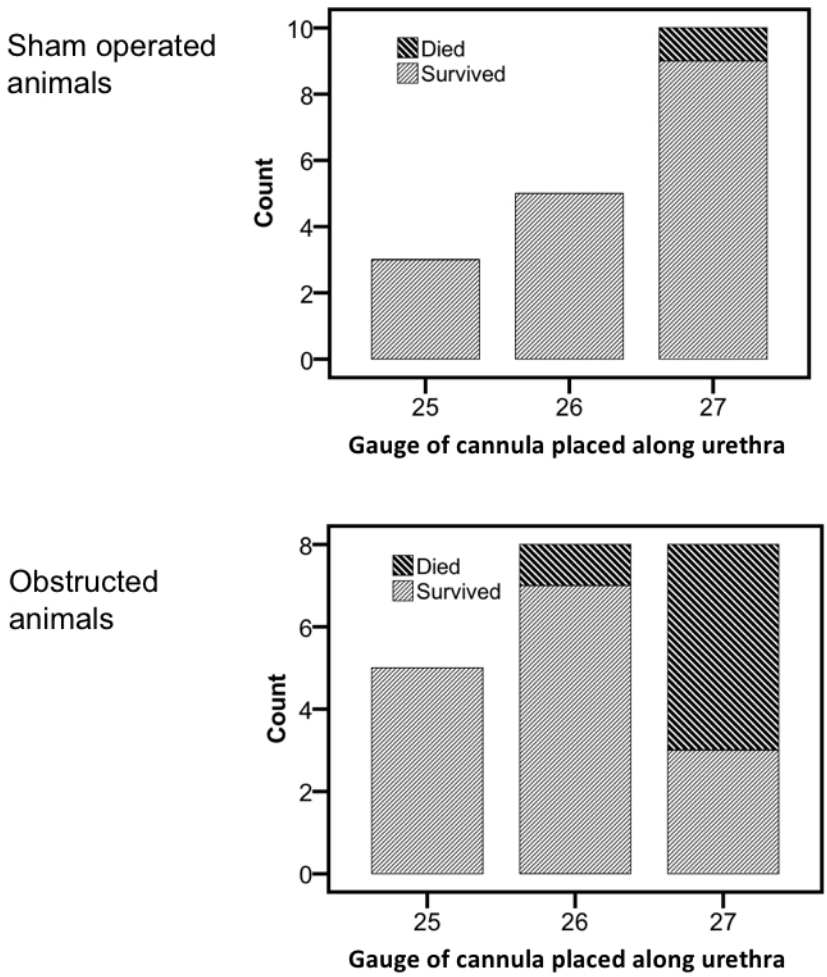

Figure 3. Mortality Increases with Degree of Partial Obstruction. One out of 18 mice died after sham NeMO procedure. When using a 25-gauge cannula as a placeholder along the urethra, all 5 operated mice survived, while 1/8 or 5/8 mice died when using a 26-gauge or 27-gauge cannula, respectively (p = 0.039, Fisher's exact test).

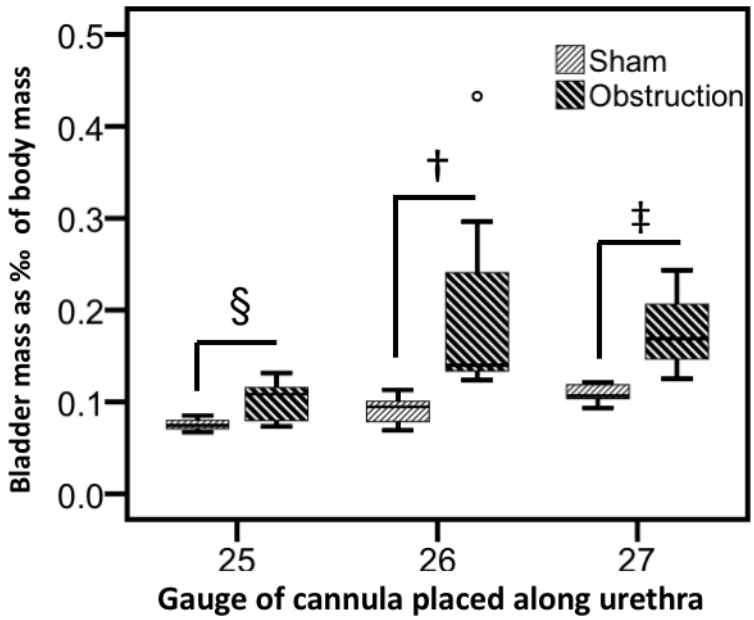

Figure 4. 26-gauge and 27-gauge Placeholder during NeMO Result in Effective Increase in Relative Bladder Mass after 2 Weeks. NeMO in female mice using a 25-gauge cannula did not result in a significant increase in relative bladder mass (**§**); when an only 26-gauge cannula was used, an over 2-fold increase in relative bladder mass occurred (p = 0.04; **†**); obstructions with a 27-gauge placeholder resulted in an over 60% increase in relative bladder mass (p = 0.004; **‡**).

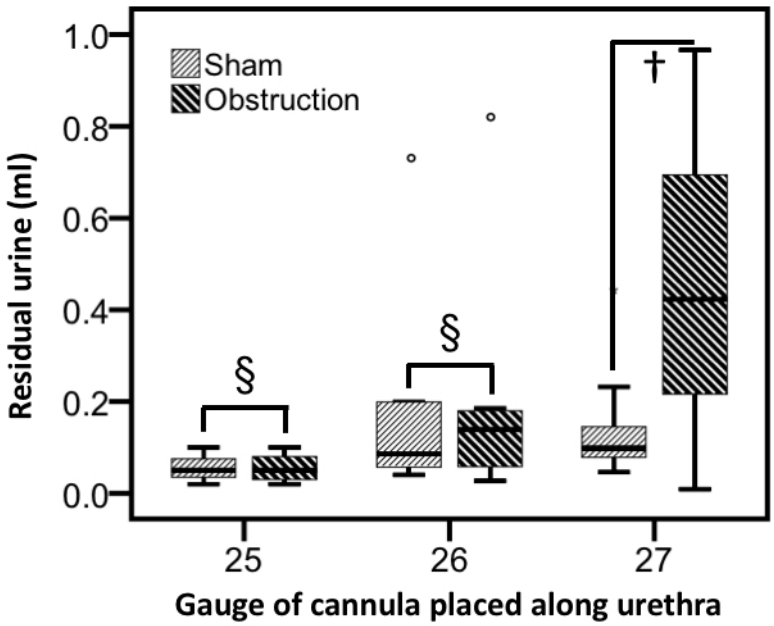

Figure 5. Maintained Bladder Emptying 2 Weeks after NeMO. Using a 25-gauge or a 26-gauge cannula along the urethra during NeMO procedure did not result in a change in residual urine after 2 weeks (**§**). The 3-fold increase in mean residual urine volume in the 27-gauge group compared to shams did not reach statistical significance (obstructed n=3; **†**).

Discussion

Animal mortality is one of the major concerns of the traditional model of pBOO at the proximal urethra, at 15% or more in many reports[10,11,13]. NeMO appears to have minimal mortality when applied in female rats. Proximal urethral obstruction in mice is technically more challenging than in rats and thus even more prone to complications. Using a 26-gauge cannula as a placeholder for our mid-urethral approach, we had only minimal animal mortality. In an ongoing study where we applied NeMO in female mice using a 26-gauge cannula only 2 out of 18 mice were over-obstructed (data not shown). Using a smaller placeholder such as a 27-gauge cannula over half of the obstructed mice died due to over-

obstruction with urinary retention or bladder rupture. Therefore, we consider a 26-gauge placeholder to be a reasonable caliber for female mice of about 18 g.

Increase in bladder mass is one of the hallmarks of pBOO in human disease. We identified NeMO to effectively induce a significant increase in bladder mass after only 2 weeks in rats as well as in mice. Average increase in bladder mass after pBOO at the proximal urethra is over 2.5-fold after 2 weeks and hence much higher than what we observed after NeMO[12]. Such an excessive increase in organ mass however does not reflect the majority of infravesical obstructive uropathies which researchers aim to model when applying pBOO; in this regard, NeMO offers a less invasive and well tolerated pBOO model with a more moderate remodeling-response that is much closer to human pathology than the traditional proximal pBOO. The more pronounced increase in bladder mass in proximal pBOO is most likely related to a denervation injury resulting from dissection around the bladder neck; denervation then may jeopardize detrusor-sphincter crosstalk and cause a functional outlet obstruction even in sham operated animals[12].

Residual urine was also significantly increased in female rats undergoing NeMO after only 2 weeks. In mice on the other hand, only using the 27-gauge cannula as a placeholder tended to increase the amount of residual urine in mice. However, while rats virtually all void when placed under gaseous anesthesia, we did not observe this in mice where only very few voided when induced. This non-voiding behavior of mice probably masks the true amount of residual urine and necessitates another stimulus to induce micturition in future studies.

While the **degree of obstruction** using a 0.9 mm rod along the urethra in female rats seems to be appropriate, combining minimal animal mortality with a clear increase in bladder mass and residual urine, calibrating the degree of obstruction for NeMO in female mice is more challenging. On one hand, using a 25-gauge cannula did not lead to a detectable increase in bladder mass, using the 27-gauge cannula on the other hand resulted in over-obstruction in more than half of the mice undergoing pBOO. Only the 26-gauge cannula had a reasonable response in terms of organ growth along with a low animal mortality.

Since pBOO is clearly more prevalent in men than in women, using male animals would be preferable when modeling human pBOO. Our nerve sparing mid-urethral obstruction (NeMO) technique obviously does not address this shortcoming of accepting animals' hormone status influencing the inflammatory response that initiates tissue remodeling. The low variability and low animal mortality of NeMO in mice, however, will make investigation of bladder remodeling amenable to the full spectrum of transgenic methods, which are very limited in larger rodents.

In summary, NeMO for female rats and female mice is easy to do, has a low animal mortality, and avoids the denervation injury to the bladder resulting from dissection around the bladder neck.

Disclosures

The authors have nothing to disclose.

Acknowledgements

The work was funded by a CHIR operating grant (DJB).

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