Reviewer's report

Title: Removal of urothelium affects bladder contractility and ATP but not NO release in rat urinary bladder

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Reviewer: Russ Chess-Williams

Reviewer's report:

1. The manuscript is well written, the data sound and appropriate statistical analysis has been performed. The study is also attempting to answer an important question: where are ATP and NO released from in the bladder? The approach is novel in trying to remove the urothelial layer of the bladder by "swabbing" and hopefully leaving the suburothelium intact. The responses of intact and "swabbed" tissues were different suggesting that ATP and NO release may be from different regions of the bladder, but the big problem is identifying which regions have actually been studied. The urothelium is a thin layer of epithelial cells on the inner surface of the bladder and "swabbing" is intended to remove just this thin layer leaving the suburothelium and smooth muscle intact. However figure 1 appears to show that "swabbing" removes both the urothelial and the suburothelial layers right down to the smooth muscle. If this is correct, it will have serious implications for all the conclusions that have been made. If there is further evidence that swabbing does not remove the suburothelium, then this could be presented. If there is no further evidence for this, then I would recommend that the manuscript be revised to indicate that intact versus smooth muscle layers have been examined, and assume that the suburothelium has also be removed during the "swabbing" process. The conclusions are still of interest but rather than claim NO comes from the deep layers of the suburothelium, it can be concluded that it comes from the smooth muscle. This change would also need to be made to the title.

2. I found the asterix in each of the figures 4, 5 & 6 a bit confusing as they are placed on the only columns that are not increased by stimulation. It would be less confusing to place the asterix on the values that are significantly changed. As it stands, there is also no explanation of the asterix in either the figure or the legend.

3. I do not know how cross-sectional area (it appears as “transactional are” in the manuscript) of a strip can be calculated from it’s weight and length? Is it possible to add this to the methods?

4. Reference 11 is a paper on ATP release but it is used on page 9 as a reference for reduced contraction induced by the urothelium. This original article should be cited and not another paper that only refers to the original paper.

5. Figure 2 – it would be nice to see the concentrations of drugs used stated in
the figure or in the legend.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'