Open peer review report 1

Reviewer: David Parker, University of Cambridge, UK.

Comments to the authors:

This article tries to do what many other articles have tried for many years, to promote regeneration. It takes a different approach, but the rationale uses the same logic, promote regeneration as this will equal recovery. This seems logical given that the effects of spinal injury are due to the removal of connections across the lesion site, but it begs the question to claim that regeneration equals recovery (Parker 2017). And despite many studies showing the ability of making axons regenerate across lesion sites, this has not translated into any useful clinical approach (Steward et al 2012).

The authors claim in several places that their approach has resulted in motor recovery, but the BBB score suggests a very modest improvement above control, and certainly nowhere near behavioural recovery. At a minimum they need to be clear and direct on this, claiming recovery from what looks a significant increase in the locomotor score. To someone who doesn't know what the rating scale of the behavioural test means it would be easy to be convinced that this is a significant improvement, or recovery, of motor function, but what this statistically significant improvement means in terms of a significant recovery should be made very clear. In reality the treated group show a very modest functional improvement, not "motor recovery" (line 17). The authors should say what a BBB score of 8 means functionally.

They also cannot rule out that the recovery they see could be due to other changes (e.g. stronger reflex responses below the lesion site). They, like many others, use a second lesion to show that this abolished recovered function and thus that this recovery must reflect the regenerated inputs that were removed by the second lesion. This is again logically incorrect. The regenerated inputs may be a necessary aspect of the recovery, but this doesn't mean that it is sufficient on its own to explain the modest recovery seen. In any integrated system (i.e. the normal and lesioned spinal cord), multiple factors will be involved in any functional output. The regeneration they see will be one of these factors, but that does not mean that it is the only one. They have not attempted to study other aspects. At a minimum they need to address the possibility that sub and supra-lesion changes may contribute to the modest improvement seen, and ideally show evidence that the regeneration is the most significant factor.

The statistical analysis needs further detail. For example, are sample sizes sufficient as claimed in passing (line 86) but this isn't documented or explained later. Some comment on whether the group of 10 animals in each condition is sufficiently powered would be needed. Also, are parametric tests appropriate for the ordinal BBB scale used? This has been discussed in the literature, and some comment is needed here to justify the statistical approach.

The experimental approach also needs more detail. In the clean transection experiments the cleanness of the cut is an important factor in reducing oedema etc. Were the animals in the untreated and the treated group known to the person doing the surgery and thus open to unconscious bias? Severance was performed with a surgical blade (line 106), but was this done by hand? Was there any difference in the cleanness of the cut in different animals (and how was this assessed?), something that is hard to standardise if done by hand, that could contribute to the differences in score between the treated and untreated groups? How soon after the transection was the PBS or TexasPEG applied? Signs of Wallerian degeneration in these clean cut appositions are seen within minutes, and to help reduce this the cut ends need to be apposed as soon as possible. Was their any difference in the delay in the treated and untreated group? Further detail on these methods are needed.

Line 75, "electropositionally". What does this mean?

Line 230. A reference is needed for the claim that 5-20% regeneration is sufficient.

Line 238. What do you mean by a very thin layer of interneurons? That the damage is restricted compared to a contusion injury?

The reference to Kim et al (2016b) says submitted. I assume a decision has been made on a paper submitted in 2016?