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Commentary

Reproducibility – The key towards clinical implementation of spinal cord injury treatments?

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Axons fail to regenerate after spinal cord injury (SCI). SCI is a severe condition resulting in livelong disability due permanent disruption of connectivity between the brain and the rest of the body (Hilton and Bradke, 2017). Researchers have implemented different strategies to promote axon regeneration by targeting intracellular neuronal processes underlying axon growth or reducing inhibition by extracellular factors (Bradbury et al., 2002; Curcio and Bradke, 2018; Schwab and Strittmatter, 2014). Despite numerous published preclinical studies with new targets to treat SCI, no regenerative therapy is available to patients yet and the main clinical option to promote functional improvements remains rehabilitation therapy (Dietz and Fouad, 2014). Why do regenerative therapies that show promise in preclinical models of SCI fail to make a difference clinically? One of the major issues is the lack of robustness and reproducibility in preclinical studies, including in SCI research (Steward et al., 2012).

In order for a preclinical study to make it to the clinical stage it should ideally be replicated independently. A confirmatory replication provides confidence that a particular therapeutic approach is efficacious. To reproduce a study, a different lab tries to completely or partially replicate a previous study. Although the aim is to fully replicate the experimental methodology of a previous study, in practice this is almost impossible. Therefore, often only key aspects of the original study are replicated. In SCI research, replication studies are rarely performed due to a lack of funding, the absence of recognition researchers get for performing such studies or problems with publishing. In light of these concerns, the “Facilities of Research Excellence-Spinal Cord Injury” (FORE-SCI) program was developed to replicate key studies in the SCI field (Steward et al., 2012). A number of studies were carefully selected due to critical criteria such as clinical relevance, endpoints, clinical translatability, effect size, scientific quality of the publication, and general strengths and weaknesses. The reality hit hard that out of 12 studies only one study was fully replicated, six were not reproducible and the rest had mixed results or there was only partial replication.

Replication studies are challenging to conduct. Not only the way a drug is administered but also the lesion model, the animal strain, as well as the lesion surgery and the quality of outcome measures influence reproducibility. Behavioral analyses for example are prone to subjectivity and alter not only from lab to lab, but also from person to person or from timing of behavioral assessment. Therefore, every successful and non-successful reproduction enhances our understanding of possible SCI treatments. And those studies are important for the clinical progress in the field.

Notably, treatment of SCI with the microtubule stabilizing drugs called Epothilone B and Epothilone D has received attention due to the verification of functional improvement after SCI of two independent labs (Ruschel et al., 2015; Ruschel and Bradke, 2018; Sandner et al., 2018). This could have a great impact and push the use of Epothilones towards a clinical translation since this drug is already FDA approved and is administered systemically and therefore non-invasive (Vagnozzi and Silver, 2018). In addition, an earlier study investigating Taxol which has the same microtubule stabilizing effect as Epothilone was replicated within the FORE-SCI program by Popovich and colleagues (Hellal et al., 2011; Popovich et al., 2014). This replication study was partly successful by showing a reduction of fibrotic scarring and it clearly demonstrated the importance of communication between the two labs. The way Taxol was administered played a crucial role on its effect.

In their recent publication in *Experimental Neurology*, Oudega and colleagues replicated a study from Yang et al. (2015) in a rigorous blinded manner with striking robustness in close cooperation between their group in Miami and the original lab in Beijing (Oudega et al., 2019). In the original study, the authors propose a new strategy to improve regeneration after SCI: implanting a scaffold filled with chitosan slowly releasing neurotrophin 3 (NT3) (Yang et al., 2015). The NT3-chitosan implant promoted neurogenesis in the spinal cord, which facilitated electrophysiological signaling across the complete lesion and improved functional recovery. Three main groups to analyze outcome measures were chosen. After complete transection and removal of a 5 mm piece of the thoracic spinal cord, a chitosan tube slowly releasing NT3 was implanted into the spinal cords of one group with comparison to control groups without treatment or with an empty chitosan tube implanted. One year after lesion, newly formed neuronal tissue was only found in the NT3-chitosan treated group, whereas there was little tissue formation in the empty tube group. In the lesion only group, the gap was filled with scar like and cystic tissues. The neuronal tissue in

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the NT3-chitosan group led to a functional improvement tested with the BBB score from 4 weeks after injury up to a ten times increase by one year after injury (Basso et al., 1995). In the control groups, the score was not higher than 1.5 one year after injury, with a score of 1 corresponding to slight movement of one or two joints. Furthermore, electrophysiological signals were only recorded in the treated group at several time-points after lesion. In an additional study, the investigators proposed an underlying mechanism based on a transcriptional analysis of the injured spinal cord which revealed enhanced vascularization and a suppressed inflammatory immune response in the NT3-chitosan treated groups (Duan et al., 2015).

In the replication study, Oudega and colleagues chose to repeat the transection-only group, the group with the NT3-chitosan implant and added laminectomy-only as an additional control group. They analyzed the effect of the NT3-chitosan implant three month after injury and validated the behavioral, electrophysiological and anatomical results. Altogether they found that treatment with the NT-3 releasing chitosan tube enhanced motor function, electrophysiological signals across the lesion and the formation of neuronal tissue, replicating these outcome measures of the original study. The experiments were carefully designed to undergo a blinding strategy; specifically, rats were identified with microchips. All outcome measures including functional, electrophysiological and anatomical assessment were performed blinded. Only for analysis, rats were sorted into their respective groups.

The overall success of the replication is based on the close interaction with the group of the original study. Nevertheless, looking in detail not all data were reproduced completely. The results of the BBB score were lower in the replication study compared to the original study. In the original study the NT3-chitosan group showed an improvement of mean BBB score at four weeks with a steady rise for up to a year. One year post injury the BBB score was 11.4–12.2, corresponding to frequent to consistent weight supported plantar stepping of the hindlimbs. In the replication study, the rats were only tested at 3-month post injury. At that time-point, the control group without a chitosan scaffold remained at a score around 0 and the laminectomy only group had a BBB score of 20, which indicates almost normal walking. The NT3-chitosan group had a mean BBB score of 2.0, with extensive movement of one joint or extensive movement of one joint and slight movement of one other joint. At this time point, the original study reported a score above 7, respective to extensive movement of all three joints. Nevertheless, the groups in Beijing and Miami confirmed independently, that the treated group performed better in overground walking than the transection-only group.

Generally speaking, replication of behavioral data is complicated and the robustness is low. That means small changes in the behavioral assessment can have a big impact on the outcome. For example, the rats were housed under different conditions (in groups) as compared to single housing in the original study. Moreover, the replication study only tested behavioral outcome at three months post-injury, whereas in the original study hindlimb motor function was tested weekly. However, the overall outcome that hindlimb function could be enhanced to some extent is in accordance with the original data. Altogether, the replication was a rare success and therefore holds great promise in the future for the use of an NT3-chitosan treatment.

Recently, the Li lab could provide more evidence of the effectiveness of this treatment and went a step closer towards a clinical study: the NT3-chitosan treatment has also proven effective in a monkey model of SCI (Rao et al., 2018). This study investigated the effect of the NT3-chitosan implant in hemisectioned monkeys and the authors observed striking long-distance axon regeneration across the lesion and functional improvement.

It will be interesting to see whether the treatment will further develop and be supported in a clinical stage. The NT3-chitosan treatment is an invasive procedure. People with spinal cord injuries would have to undergo surgery which in the end could harm more than it helps. This treatment ultimately promotes endogenous stem cell proliferation. However, compared to other attempts of promoting regeneration by implanting exogenous stem cells, this method may have a reduced risk of side effects like tumor formation (Assinck et al., 2017).

The current study underlines the importance of replicating studies in the spinal cord injury field. These studies are underestimated and should be supported more. To aim at the main goal to help people with spinal cord injuries and to bring developments in preclinical research to a clinical trial, it is a must to invest in this kind of studies.

In summary, the study by Oudega and colleagues is a very carefully planned and time-consuming study that relied on direct communication and interaction among the two labs. Thereby, the study managed to replicate previous findings in the field. In the future, these findings should be taken as an example and motivate other scientist to reproduce data which could have a clinical implication but also to share data and make preclinical SCI research more robust (Callahan et al., 2017).

References

Assinck, P., Duncan, G.J., Hilton, B.J., Plemel, J.R., Tetzlaff, W., 2017. Cell transplantation therapy for spinal cord injury. Nat. Neurosci. 20, 637–647. https://doi.org/10.1038/nn.4541.

Basso, D.M., Beattie, M.S., Bresnahan, J.C., 1995. A sensitive and reliable locomotor rating scale for open field testing in rats. J. Neurotrauma 12, 1–21. https://doi.org/10.1089/neu.1995.12.1.

Bradbury, E.J., Moon, L.D.F., Popat, R.J., King, V.R., Bennett, G.S., Patel, P.N., Fawcett, J.W., McMahon, S.B., 2002. Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416, 636–640. https://doi.org/10.1038/416636a.

Callahan, A., Anderson, K.D., Beattie, M.S., Bixby, J.L., Ferguson, A.R., Fouad, K., Jakeman, L.B., Nielson, J.L., Popovich, P.G., Schwab, J.M., Lemmon, V.P., 2017. Developing a data sharing community for spinal cord injury research. Exp. Neurol. 295, 135–143. https://doi.org/10.1016/j.expneurol.2017.05.012.

Curcio, M., Bradke, F., 2018. Axon regeneration in the Central Nervous System: facing the challenges from the inside. Annu. Rev. Cell Dev. Biol. Annu. Rev. Cell Dev. Biol 34, 495–521. https://doi.org/10.1146/annurev-cellbio-100617.

Dietz, V., Fouad, K., 2014. Restoration of sensorimotor functions after spinal cord injury. Brain 137, 654–667. https://doi.org/10.1093/brain/awt262.

Duan, H., Ge, W., Zhang, A., Xi, Y., Chen, Z., Luo, D., Cheng, Y., Fan, K.S., Horvath, S., Sofroniew, M.V., Cheng, L., Yang, Z., Sun, Y.E., Li, X., 2015. Transcriptome analyses reveal molecular mechanisms underlying functional recovery after spinal cord injury. Proc. Natl. Acad. Sci. 112, 13360–13365. https://doi.org/10.1073/pnas.1510176112.

Hellal, F., Hurtado, A., Ruschel, J., Flynn, K.C., Laskowski, C.J., Umlauf, M., Kapitein, L.C., Strikis, D., Lemmon, V., Bixby, J., Hoogenraad, C.C., Bradke, F., 2011. Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury. Science 80 (331), 928–931. https://doi.org/10.1126/science.1201148.

Hilton, B.J., Bradke, F., 2017. Can injured adult CNS axons regenerate by recapitulating development? Development 144, 3417–3429. https://doi.org/10.1242/dev.148312.

Oudega, M., Hao, P., Shang, J., Haggerty, A.E., Wang, Z., Sun, J., Liebl, D.J., Shi, Y., Cheng, L., Duan, H., Sun, Y.E., Li, X., Lemmon, V.P., 2019. Validation study of neurotrophin-3-releasing chitosan facilitation of neural tissue generation in the severely injured adult rat spinal cord. Exp. Neurol. 312, 51–62. https://doi.org/10.1016/j.expneurol.2018.11.003.

Popovich, P.G., Tovar, C.A., Lemeshow, S., Yin, Q., Jakeman, L.B., 2014. Independent evaluation of the anatomical and behavioral effects of Taxol in rat models of spinal cord injury. Exp. Neurol. 261, 97–108. https://doi.org/10.1016/j.expneurol.2014.06.020.

Rao, J.-S., Zhao, C., Zhang, A., Duan, H., Hao, P., Wei, R.-H., Shang, J., Zhao, W., Liu, Z., Yu, J., Fan, K.S., Tian, Z., He, Q., Song, W., Yang, Z., Sun, Y.E., Li, X., 2018. NT3-chitosan enables de novo regeneration and functional recovery in monkeys after spinal cord injury. Proc. Natl. Acad. Sci. 115, E5595–E5604. https://doi.org/10.1073/pnas.1804735115.

Ruschel, J., Bradke, F., 2018. Systemic administration of epothilone D improves functional recovery of walking after rat spinal cord contusion injury. Exp. Neurol. 306, 243–249. https://doi.org/10.1016/j.expneurol.2017.12.001.

Ruschel, J., Hellal, F., Flynn, K.C., Dupraz, S., Elliott, D.A., Tedeschi, A., Bates, M., Sliwinski, C., Brook, G., Dobrindt, K., Peitz, M., Brustle, O., Norenberg, M.D., Blesch, A., Weidner, N., Bunge, M.B., Bixby, J.L., Bradke, F., 2015. Axonal regeneration. Systemic administration of epothilone B promotes axon regeneration after spinal cord injury. Science 80 (348), 347–352. https://doi.org/10.1126/science.aaa2958.

Sandner, B., Puttagunta, R., Motsch, M., Bradke, F., Ruschel, J., Blesch, A., Weidner, N., 2018. Systemic epothilone D improves hindlimb function after spinal cord contusion injury in rats. Exp. Neurol. 306, 250–259. https://doi.org/10.1016/j.expneurol.2018.01.018.

Schwab, M.E., Strittmatter, S.M., 2014. Nogo limits neural plasticity and recovery from injury. Curr. Opin. Neurobiol. 27, 53–60. https://doi.org/10.1016/j.conb.2014.02.011.

Steward, O., Popovich, P.G., Dietrich, W.D., Kleitman, N., 2012. Replication and reproducibility in spinal cord injury research. Exp. Neurol. 233, 597–605. https://doi.org/10.1016/J.EXPNEUROL.2011.06.017.

Vagnozzi, A.N., Silver, J., 2018. Targeting the cytoskeleton with an FDA approved drug to promote recovery after spinal cord injury. Exp. Neurol. 306, 260–262. https://doi.org/10.1016/J.EXPNEUROL.2018.05.007.

Yang, Z., Zhang, A., Duan, H., Zhang, S., Hao, P., Ye, K., Sun, Y.E., Li, X., 2015. NT3-chitosan elicits robust endogenous neurogenesis to enable functional recovery after spinal cord injury. Proc. Natl. Acad. Sci. 112, 13354–13359. https://doi.org/10.1073/PNAS.1510194112.