# Absence of toxicity in killer proteins

**Ilona Sergeeva**
Lake Forest College
Lake Forest, Illinois 60045

*Toxicity in a prion-infected brain has long been assumed to be present because of infectious prion proteins. New methods were used to test this assumption.*

Throughout its history, humanity has dealt with countless wars, pandemics, natural disasters, and other such cataclysms. In recent decades, a new challenge arose—prions. Prion proteins inhibit brain activity and aid neuron connections with their healthy form: $PrP^{C}$ (Prion Protein Cellular). However, a misfolding of such protein ($PrP^{Sc}$ - Prion Protein Scrapie) leads to irreversible neurodegeneration, or continuous damage to the brain, inevitably leading to death. This infectious disease involves many types, two famous ones being Creutzfeldt-Jakob disease (CJD) and scrapie, all presenting the characteristics of brain damage and loss of neuronal cells. The mechanisms of each type are still heavily studied today; however, the cure is not yet found. It has long been assumed that the infectivity of prions is associated with neurotoxicity and is the cause of their fatality. Benilova and colleagues (2020) researched this theory using mice and new methods that allow the separation of infectivity and neurotoxicity.

In 1967, it was proposed that proteins were infectious and were involved with scrapie. Two decades later, Stanley Prusiner discovered proteins he later called “prions” from scrapie-infected hamster brains. He identified prions as infectious particles that lack nucleic acid (Prusiner, 1998). $PrP^{C}$ was believed to be infectious and neurotoxic, leading to its inevitable danger; however, more and more evidence suggests that that is not the case (Ma et al., 2002). Neurotoxicity refers to a negative effect on the nervous system by proteins or chemicals (Spencer and Lein, 2014). Neurotoxic disorders progress during or right after exposure, and the assumption that neurotoxicity was present in prion proteins was under question.

After the discovery that the spread of prions proceeds in two phases, first exponentially and then inversely proportional to $PrP^{C}$ concentrations (Sandberg et al., 2014), Benilova and their colleagues (2020) hypothesized that prions were not neurotoxic themselves, but it is rather the pathway switch in the second phase of prion propagation that causes this neurotoxicity. To test this hypothesis, prions of Rocky Mountain Laboratory (RML) infected mice were isolated and the infectivity was tested by the Automated Scrapie Cell Assay (ASCA) (Schmidt et al., 2015). This assay incorporates cells exposed to infected brain homogenates cultures and splits them up several times. The number of “spots” was determined and explained by $PrP^{Sc}$ progression. The more misfolded prion proteins were present, the more infectious the cell culture is. The ASCA specifically requires special equipment and is able to analyze a larger number of samples (Klohn et al., 2003).

Neurites are axons and dendrites extending from a neuron’s cell body (Xiao et al., 2013). Their retraction is identified as the shortening of the microtubule skeleton (Prager-Khoutorsky and Spira, 2009). The toxicity of samples was tested by viewing neurite retraction, as neurite degeneration can be used to measure neurotoxicity in the brain and spinal cord (Dixon and Philber, 2015). Neurite beading, for instance, or the increased concentration of organelles along neurites, signifies neurotoxicity in some neurodegenerative diseases. The more retraction observed, the more toxicity was present. Brain homogenate of normal, uninfected mice and RML prion-infected mice were compared for neurite retraction.

After examining the prion rods from the RML brain homogenate, no neurite retraction was found, indicating no neurotoxicity (Fig. 1). This data disproved the previous assumption concerning prion neurotoxicity and supported the first part of the researchers’ hypothesis (i.e., prions not being toxic).

But what about the infectivity vs. the neurotoxicity of prions? The proteins must be deadly because of neurotoxicity—or so previously thought. After the first discovery of the absence of neurotoxicity in prions, the researchers moved on to the next big question: is prion infectivity caused by neurotoxicity?

To find the answer, the researchers decided to see how complete neurotoxicity removal would affect the infectivity of the prion-infected brain. In theory, if the two are linked, abolishing neurotoxicity will get rid of infectivity. For this procedure, sarkosyl, a mild detergent used for denaturing proteins (Thibeault et al., 2019), was used to remove neurotoxicity with the previous knowledge that sarkosyl has no effect on prion infectivity. Treatment of RML-infected brain homogenate with the detergent revealed that neurotoxicity was abolished entirely. Remarkably, infectivity, measured by the ASCA, was not at all decreased (values measuring infectivity were not significantly different) (Fig. 1). If anything, infectivity was slightly increased after neurotoxicity was removed. These findings strongly support the claim that the neurotoxicity of the brain homogenate has no effect on prion infectivity.

These new connections are huge discoveries in prion disease research. This evidence is solid enough to disregard previous claims. A lot of unanswered questions still remain, and such results shine a light on new challenges. For example, identifying the cause(s) of neurotoxicity in the prion-infected brain if prion proteins are not responsible. Overall, this is a major breakthrough that sets a direction for future research.

More studies need to be done to replicate this experiment and to identify the neurotoxic species responsible for the toxicity of prion-infected brain homogenate as well as paint a clearer picture of the basis of prion neurotoxicity. The finish line to understanding prion propagation and infectivity is still far off, but this connection is another step closer to uncovering the mysteries of one of the most aggressive neurodegenerative diseases.

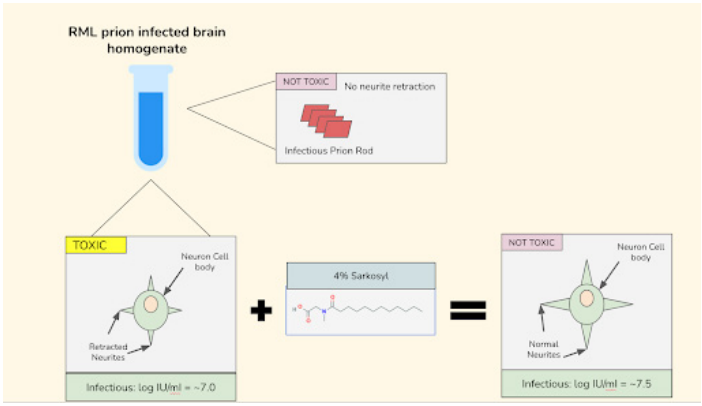


**Figure 1.** Testing of prion neurotoxicity. Benilova et al. (2020) completed two major experiments, firstly identifying the toxicity of prion rods in infected brain homogenate. After observing no neurite retraction, it was concluded that the prion rods themselves were not neurotoxic, even though the brain homogenate was. Going deeper, neurotoxicity of the brain homogenate was abolished with the addition of 4% sarkosyl. Neither neurite retraction nor fragmentation was observed, meaning neurotoxicity was not present. The ASCA identified the brain homogenate as infectious before and after the addition of sarkosyl. The researchers used these models to correlate the infectivity and neurotoxicity of the brain homogenate.

## References

Benilova, I., Reilly, M., Terry, C., Wenborn, A., Schmidt, C., Marinho, A. T., . . . Collinge, J. (2020). Highly infectious prions are not directly neurotoxic. Proceedings of the National Academy of Sciences 117(38), 23815-23822.
Dixon, A. R., and Philbert, M. A. (2015). Morphometric assessment of toxicant induced neuronal degeneration in full and restricted contact co-cultures of embryonic cortical rat neurons and astrocytes: using m-Dinitrobezene as a model neurotoxicant. Toxicology in Vitro 29(3), 564-574.
Klohn, P. C., Stoltze, L., Flechsig, E., Enari, M., and Weissmann, C. (2003). A quantitative, highly sensitive cell-based infectivity assay for mouse scrapie prions. Proceedings of the National Academy of Sciences 100(20), 11666-11671.
Ma, J., Wollmann, R., and Lindquist, S. (2002). Neurotoxicity and neurodegeneration when prp accumulates in the cytosol. Science 298(5599), 1781-1785.
Prager-Khoutorsky, M., and Spira, M. E. (2009). Neurite retraction and regrowth regulated by membrane retrieval, membrane supply, and actin dynamics. Brain Research 1251, 65-79.
Prusiner, S. B. (1998). Prions. In Prions.

Sandberg, M. K., Al-Doujaily, H., Sharps, B., De Oliveira, M. W., Schmidt, C., Richard-Londt, A., . . . Collinge, J. (2014). Prion neuropathology follows the accumulation of alternate prion protein isoforms after infective titre has peaked. Nature Communications 5(1).
Schmidt, C., Fizet, J., Properzi, F., Batchelor, M., Sandberg, M. K., Edgeworth, J. A., . . . Collinge, J. (2015). A systematic investigation of production of synthetic prions from recombinant prion protein. Open Biology 5(12), 150165.
Spencer, P.S., and Lein, P.J. (2014) Neurotoxicity. In: Encyclopedia of Toxicology, P. Wexler, ed. (Elsevier Inc., Academic Press), pp. 489–500.
Thibeault, J., Patrick, J., Martin, A., Ortiz-Perez, B., Hill, S., Zhang, S., . . . Colón, W. (2019). Sarkosyl: A milder detergent than SDS for identifying proteins with moderately high hyperstability using gel electrophoresis. Analytical Biochemistry 571, 21-24.

Xiao, Y., Peng, Y., Wan, J., Tang, G., Chen, Y., Tang, J., . . . Shi, L. (2013). The atypical guanine nucleotide exchange factor Dock4 regulates neurite differentiation through modulation of Rac1 GTPase and actin dynamics. Journal of Biological Chemistry 288(27), 20034-20045.