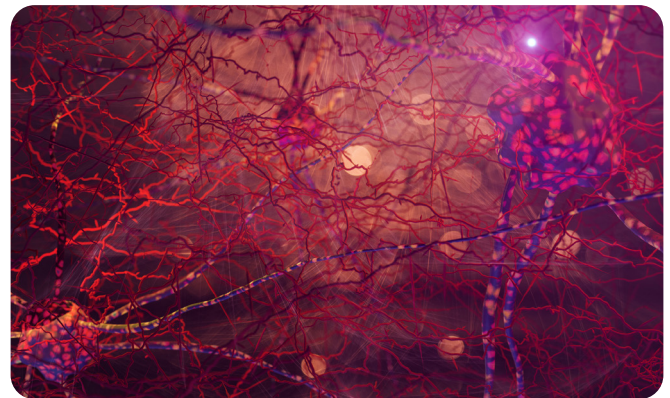


Exploring mechanisms behind alphavirus-induced post-encephalitic parkinsonism

Parkinson's disease (PD) is a neurodegenerative disorder with progressive worsening of primarily motor symptoms caused by the loss of dopaminergic neurons, which play a regulatory role in several basic brain functions, including innervating motor nerves, affecting behaviors, and sustaining working memory. The mechanism of the loss of these dopamine neurons and subsequently the loss of these neurotransmitters in the brain ecosystem is under vigorous investigation in the context of PD and has been associated with oxidative stress, neuroinflammation, and unnatural α -synuclein protein aggregation. Age, genetics, and environmental exposures – including that of infectious agents like viruses – are all risk-factors that have been implicated in neurodegenerative diseases like AD, PD or parkinsonism as well.

Several studies have reported parkinsonism in post-encephalitic viral infection survivors, such as those affected by the Spanish Flu, West Nile virus (WNV), Japanese encephalitis virus (JEV), and others; these neurological symptoms closely resemble those of PD and have been replicated to a large extent in rodent research models. The recent COVID-19 pandemic has also shown lasting neurological consequences in some patient populations, including manifestations of encephalitis. The Western equine encephalitis virus (WEEV) is among the group of viruses associated with inducing parkinsonism post-encephalitic infection, and it has been shown that its viral RNA can persist in mouse brain 3-17 months post-acute infection, pointing to potential neuroinflammatory consequences.



Dr. Bantle and Dr. Tjalkens's group recently explored this phenomenon of neuroinvasive viruses eliciting neurodegenerative symptoms of post-encephalitic parkinsonism by using bioluminescent or fluorescent recombinant WEEV in an *in vivo* mouse model following non-lethal encephalitic infection to tease the mechanism by which they may be causing lasting neurological events, even after clearing the infection at 8 weeks upon receiving passive immunotherapy.

They showed that their recombinant WEEV system – McFly – showed robust luciferase activity (Figure 1) and apparent neurobehavioral and motor deficits by 24 hrs post-infection, where mice were unable to survive more than 96 hrs due to severe encephalitic infection without immunotherapy treatment. The lab also generated McRed – a DsRed-expressing recombinant WEEV virus – to analyze viral dissemination at the microscopic level.

They showed neuronal loss of TH + dopaminergic neurons in the substantia nigra pars compacta (SNpc) compared to age-matched uninfected controls at 4 DPI by 29% using a 3D design-based stereology techniques, as well as marked activation of microglia and astrocytes in these McRed-infected brains that stained positive for IBA1 and GFAP using immunofluorescence staining.

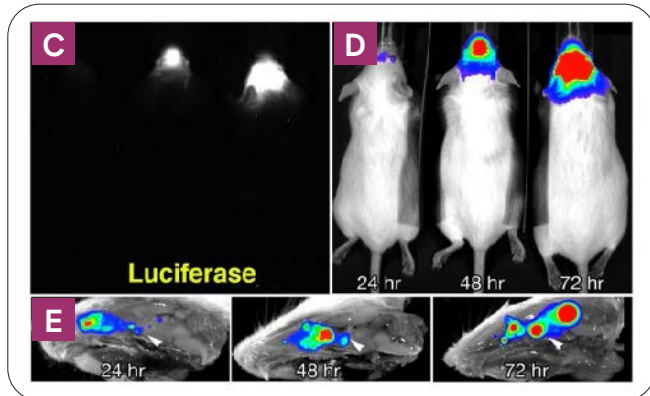


Figure 1: Taken from Figure 1, exploring the regional specificity of the recombinant WEEV post-intranasal infection in mice using *in vivo* imaging of luciferase activity (C, D) as well as *ex vivo* sagittal hemispheric imaging at various time points post-infection (E). Additional method details can be found in the method and figure legend of <https://doi.org/10.1038/s41531-019-0090-8>.

To investigate the long-term neurological consequences of infection using their recombinant system and prevent immediate mortality, inoculated mice were given a passive immunotherapy regimen to help with viral clearance to temper the spread into the midbrain and avoid severe illness, as measured by luciferase activity in a defined range of between greater than 10^5 to less than 10^6 photons/sec. Their optimized regimen saw presence of luciferase activity in the brain for up to 28 days following inoculation and no luciferase activity by 8 weeks post-infection (Figure 2). At 8 weeks, mice that had undergone immunotherapy displayed >29% loss of TH + dopaminergic neurons in the SNpc, ventral trigeminal area (VTA), and dopaminergic terminals in the striatum (ST), as well as indication of microgliosis and astrogliosis by immuno-staining; these neurological changes were associated with increased run duration and decreased duty cycling relative to control mice using quantitative gait analysis, which implied neuromotor behavioral deficit and hypokinesia associated with these losses.

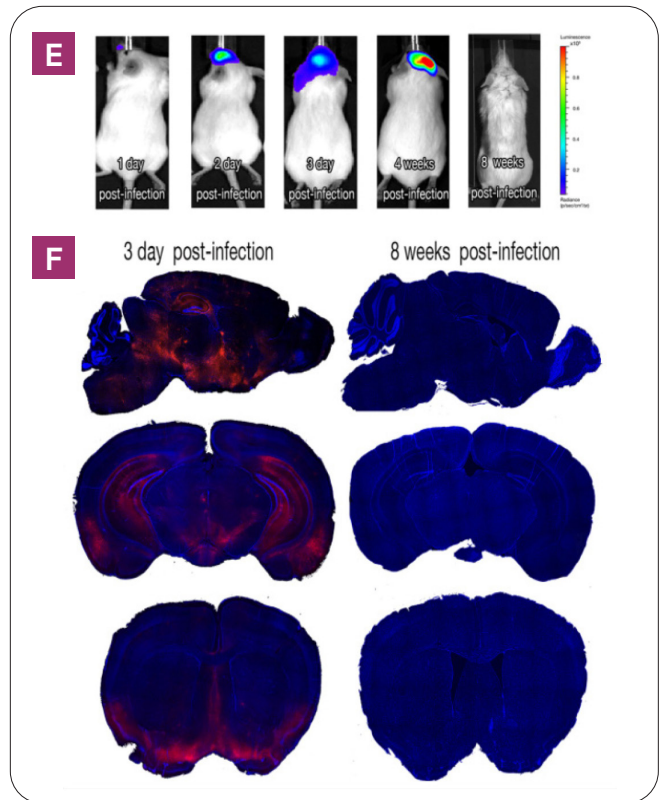


Figure 2: Taken from Figure 3, optimizing passive immunotherapy regimen to establish consistent, unlethal viral infection to the brain, with viral clearance by eight weeks post-inoculation (E,F). Additional method details can be found in the method and figure legend of <https://doi.org/10.1038/s41531-019-0090-8>.

Lastly, others have shown that α -synuclein aggregation can be induced by encephalitic infection in humans and mice. The authors explored this phenomenon in their mice model as well, noting prominent P129 + α -synuclein aggregates that co-localized mainly with IBA 1+ microglia in different regions of the brain that only appeared in mice with long-term, chronic neuroinfection and not in acutely infected mice. Select brain sections were treated with proteinase K, which showed that there were α -synuclein plaques that were insoluble and proteinase K-resistant (Figure 3). Preliminary transcriptional analysis and gene profiling using qPCR on whole-brain homogenates indicated upregulation various markers of neurodegeneration, calcium homeostasis, and mitochondrial dysfunction in these 28 DPI mice compared to the mice subjected to acute infection (3 DPI without immunotherapy).

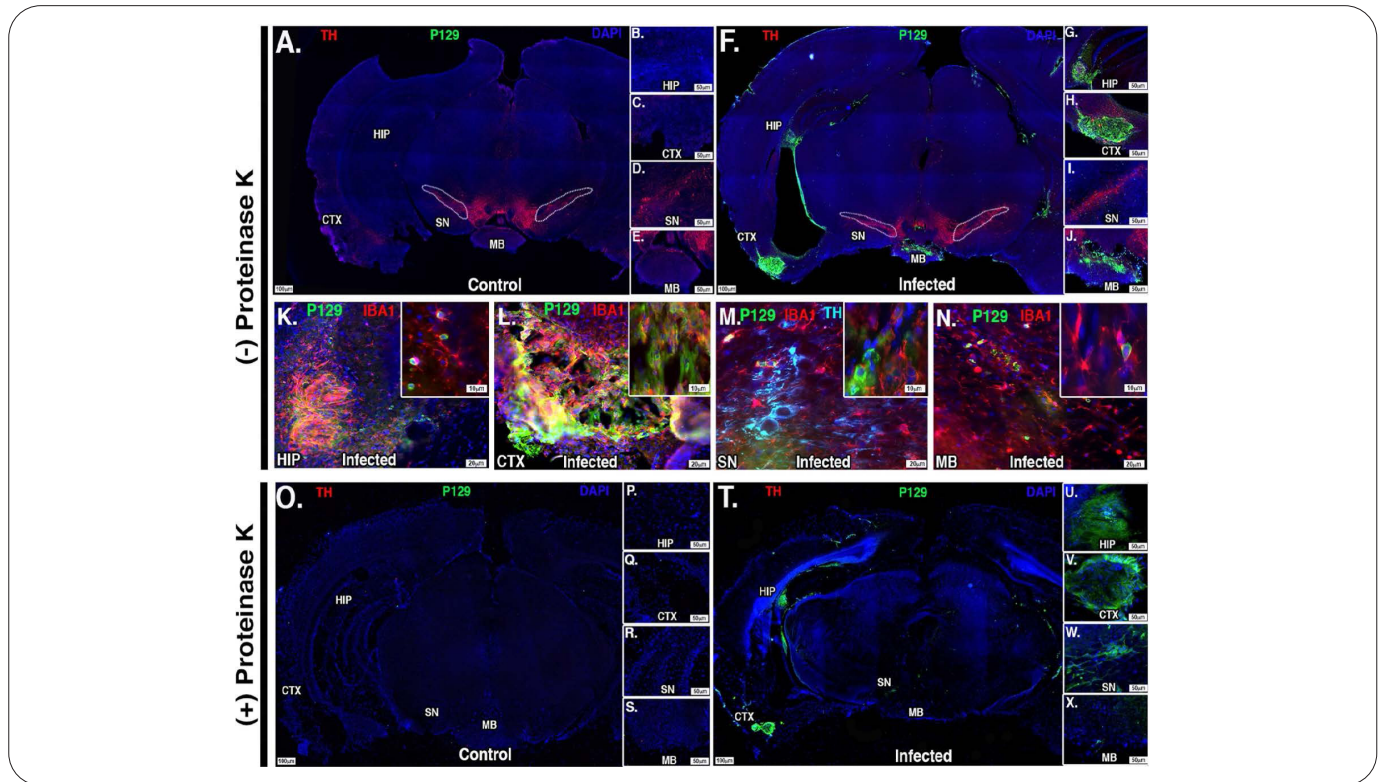
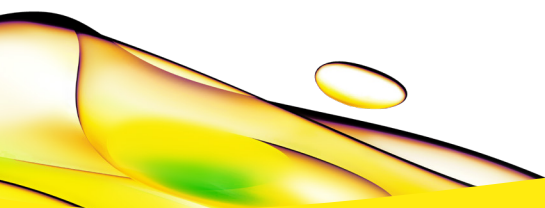


Figure 3: Taken from Figure 6, evaluating presence of proteinase-K resistant α -synuclein aggregates in McFly-infected mice on immunotherapy after 8 weeks post-inoculation compared to controls. Sections depict TH + dopaminergic neurons (red) and P129 + α -synuclein aggregates (green) (A-J), with high magnification images (B-E, G-J, K-N). Infected brain sections show proteinase K-resistant α -synuclein aggregates (green) at high and low resolution (O-X). Additional method details can be found in the method and figure legend of <https://doi.org/10.1038/s41531-019-0090-8>.

In conclusion, the authors have developed a robust animal model system employing WEEV, an alphavirus, to study viral-induced mechanisms where prolonged viral infections result in loss of dopaminergic neurons, abnormal and persistent activation of microglial or astrocyte cells, and formation of proteinase K-resistant α -synuclein aggregates, coupled with insights into neurodegenerative gene profiles. Additional research in the field is well underway to understand neurotropic viral infections and their lasting neurological consequence in the onset or progression of neurodegeneration via neuroinflammation that may be promoting neural injury in the context of not only PD and parkinsonism, but also potentially other brain or CNS disorders.

Reference

1. Bantle, C.M., Phillips, A.T., Smeyne, R.J. et al. (2019) Infection with mosquito-borne alphavirus induces selective loss of dopaminergic neurons, neuroinflammation and widespread protein aggregation. *npj Parkinsons Dis.* 5, 20. <https://doi.org/10.1038/s41531-019-0090-8> (and references included therein).



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