Using Viruses as Molecular Biology Tools: A Review of Viral Transneural Tracing

JACQUELINE WATT
Integrated Science Program, Class of 2017, McMaster University

SUMMARY

Viruses have been around for thousands of years, mastering invasion and evasion techniques of their host organisms. With the rapid progression of technology, it is these unique characteristics that can now be applied to studying cells and how they function. The most notable development is the use of neurotrophic viruses as transneural tracers. Transneural tracing traditionally involves the use of compounds that are able to pass through synapses in order to visualize connectivity between functional neurons. Neurotrophic viruses, specifically α-herpesvirus and rabies virus, have an innate ability to infect neurons and transfer between synapses. This property, along with their ability to self-amplify through replication in neurons, makes these viruses highly advantageous over traditional methods of transneural tracing. With the recent advancement of genetic engineering, there is great potential to combine genetic modification and viral transneural tracing, thus enabling more in-depth studies. This review aims to outline the unique characteristics of α-herpesvirus and rabies virus that makes them good candidates for transneural tracers and examine the potential that genetic modification can open for neuroscience research.

Received: 03/05/2017 **Accepted:** 03/30/2017 **Published:** 07/31/2017
URL: https://journals.mcmaster.ca/iScientist/article/view/1453

Keywords: transneural tracers, viruses, neural circuits, Herpesvirus, Rabies virus, viral neural transport, neurotrophic viruses, central nervous system, peripheral nervous system, motoneurons

INTRODUCTION

Manipulating and using viruses to benefit individuals and further the understanding of cells and how they function is not a new idea. Viruses are obligate, intracellular parasites that have evolved to survive within almost every species. Therefore, they provide a great opportunity to deliver treatment or study multiple species in vivo (Ugolini, 2010). This concept is especially useful when considering difficult systems to access, such as the nervous system. A revolutionary advancement in neuroscience research was the development of neurotrophic viruses as transneural tracers.

A transneural tracer is a tracer that is only able to pass through synaptically connected neurons, thus allowing for visualization of functional neurons (Callaway, 2008; Ugolini, 2010). For a tracer to be reliable there are certain requirements that must be met (Ugolini, 2010):

1. The tracer must only be able to transfer between neurons through synaptic connections.

2. The tracer should only travel in one direction through the axon.

3. The tracer must be able to label higher order neurons, meaning to pass through more than one synapse, in a neural circuit.

4. The number of synaptic connections the tracers passes through must be easily determined.

5. The tracer must be easily detectable and not degrade with time.

6. The tracer should not alter neural function.

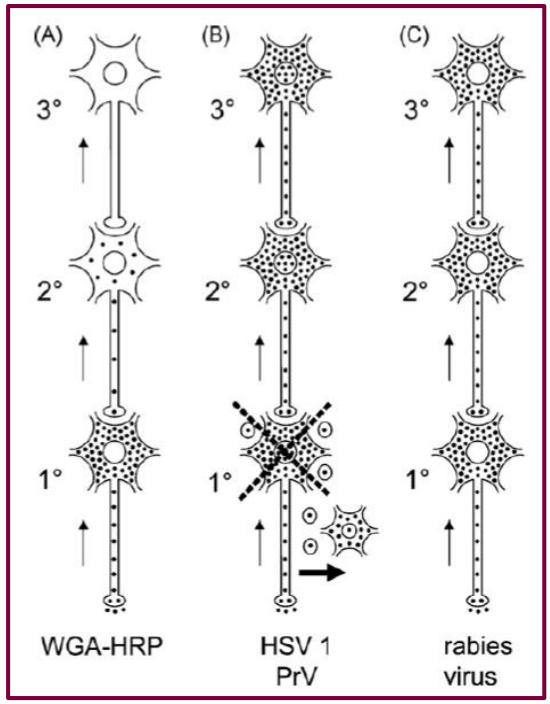

Figure 1: Diagram comparing the differences between transneural labelling with (A) conventional tracers, (B) α-herpesvirus, and (C) rabies virus. With conventional tracers only a low concentration of tracer is transferred to higher order neurons making them difficult to visualize. With viral transneural tracers a high concentration is maintained through each synaptic transfer allowing easy visualization of higher order neurons. With α-herpesvirus spurious spread is also possible (Ugolini, 2010).

Early non-viral transneural tracers, referred to as conventional tracers, included compounds that were stainable and could pass through synapses. Some examples of these compounds include horseradish peroxidase and dextran amines (Taber, et al., 2005). These compounds are weak transneural tracers as only a low concentration ever successfully crosses a synapse and consequently, the concentration is too low to label higher order neurons (Figure 1) (Ugolini, 2010). Therefore, neural circuits have to be inferred from multiple experiments showing individual synaptic connections, which is highly inefficient (Taber, et al., 2005).

Neurotrophic viruses are viruses that can enter and infect neurons. They have a natural ability to cross chemical synapses and are able to self-amplify (replicate) within each infected neuron (Callaway, 2008; Ugolini, 2010). These unique characteristics make them a much more sensitive and reliable transneural tracer, making them better than conventional tracers (Figure 1). Recently α-herpesvirus and rabies virus have been developed as transneural tracers, each with its own unique properties (Callaway, 2008; Ugolini, 2010). The purpose of this review is to discuss the properties of each of these viruses that make them useful for transneural tracing and highlight recent studies that were only successful due to these viral transneural tracers. The emerging technology of genetically modified viruses will also be discussed.

α-HERPESVIRUS

The first neurotrophic virus to be developed for transneural tracing was the α-herpesvirus, specifically Herpes Simplex virus type 1 (HSV1) and Pseudorabies virus (PrV) (Ugolini, 2010). HSV1 is able to infect and enter the central nervous system (CNS) from any injection site in all mammals, except for primates. To enter the CNS in primates, HSV1 must be injected directly into neurons of the CNS (Ugolini, 2010). PrV is a swine α-herpesvirus that can only be used as a transneural tracer for rodents and carnivores (Babic, et al., 1993 and Mettenleiter, 2003).

When considering using a virus as a transneural tracer, the virus-host interactions are crucial to the success of the study. These include what species are susceptible and permissible to the virus, how the age of the host effects the pathogenesis of the virus, and what dose and strain of the virus will be required to ensure the virus infects the desired cells. The α-herpesvirus was a good candidate for the first viral neural tracer as it is well characterised, thus these factors could be easily determined and controlled. Additionally, the number of synaptic connections can be determined using the kinetics of propagation of the virus. This would take into account the time of travel through the axon and the time to complete one replication cycle. The measurement interval for the visualization of one synaptic transfer can then be calculated for each specific strain (Ugolini, 2010). The virus is visualized using immunolabeling with florescent proteins such as green florescent protein (GFP) or red florescent

protein (RFP) (Enquist, et al., 2002). Studies have found that α-herpesvirus infection of neurons for transneural tracing results in neural degradation, thus must be limited to the onset of symptoms for ethical reasons (Ugolini, 2010).

VIRAL CHARACTERISTICS

α-herpesvirus is a large, double stranded DNA virus (Figure 2). It has a large susceptible host range; specifically, for HSV1 and PrV, humans and swine, respectively are the natural hosts, where latency is common (Tomishima, et al., 2001).

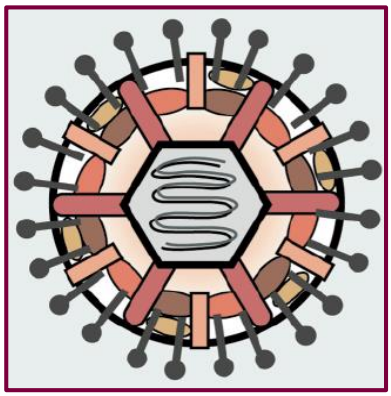

Figure 2: α-herpesvirus structure. All α-herpesvirus have a similar structure containing an icosahedral capsid, tegument, and membrane glycoproteins. The glycoprotein (gD) are illustrated as spikes on the envelope of the virion and are the part recognized by the host cell's receptors (Tomishima, et al., 2001).

α-herpesvirus enters the body through epithelial cells and then travels to the nucleus where the viral genome is released (Tomishima, et al., 2001). After initial infection, in natural hosts of the virus, α-herpesvirus can enter and remain dormant in dorsal root ganglia of the peripheral nervous system (PNS) (Enquist, et al., 2002). In this state the lytic cycle of the virus follows an alternative transcriptional program, mainly remaining transcriptionally inactive with limited expression of its genes (Ugolini, 2010). α-herpesvirus can persist in this state for the entire life of the host. However, the virus can be reactivated where it will then exit from the neuron and travel back to the original site of infection and begin actively transcribing again (Ugolini, 2010).

ENTRY INTO NEURONS

α-herpesvirus enters a neuron in the same manner as entering a cell, by plasma membrane fusion of its envelope (Tomishima, et al., 2001). The receptor for α-herpesvirus, which recognizes a glycoprotein (gD) on the outside of the virion, is speculated to be either from the tumor necrosis factor superfamily or similar to the nectin 1 and nectin 2 receptors of the immunoglobulin superfamily (Mettenleiter, 2003). From here, the capsid will travel in the axon, in retrograde motion, towards the cell body. The capsid moves along the microtubules in the axon, using the host cell's microtubule motors (Tomishima, et al., 2001). Due to the ability of the virus to reactivate, the capsid is also able to move in anterograde motion, away from the cell body, along the axon. The direction the capsid moves can be changed almost instantaneously, with the capsid having an equal probability for each direction of travel at any given time (Enquist, et al., 2002). Retrograde motion occurs approximately every one out of seven infections (Enquist, et al., 2002). This bi-directional capsid motility is an important characteristic of α-herpesvirus neural tracers as allows for varying tracing methods and techniques to be used.

Once at the cell body, the viral genes are released into the nucleus. Typically, the virus would establish latency at this point, however, when used as a transneural tracer, active replication occurs in the neuron. Assembly of the mature viral particles happens in the extra-nuclear compartments (Mettenleiter, 2003). At this point there are two pathways the virus can take (Figure 3). First, the whole virion can assemble in the extranuclear compartment. If this happens then the virus is more likely to travel in an anterograde direction along the axon (Mettenleiter, 2003). Second, the virus will not assemble into the complete viral particle and instead form sub-assemblies of the capsid, tegument, and envelope proteins (Mettenleiter, 2003). With the formation of sub-assemblies, retrograde motion along the axon is more probable, with the components accumulating at the synapse. Assembly of the whole viral particle would then occur near or at the axon terminus (Mettenleiter, 2003). The glycoprotein gE is believed to play a large role in initiating virion formation at the synapse, making it essential for transsynaptic transfer. The Us9 membrane protein, which is important for axonal localization of envelope proteins, determines

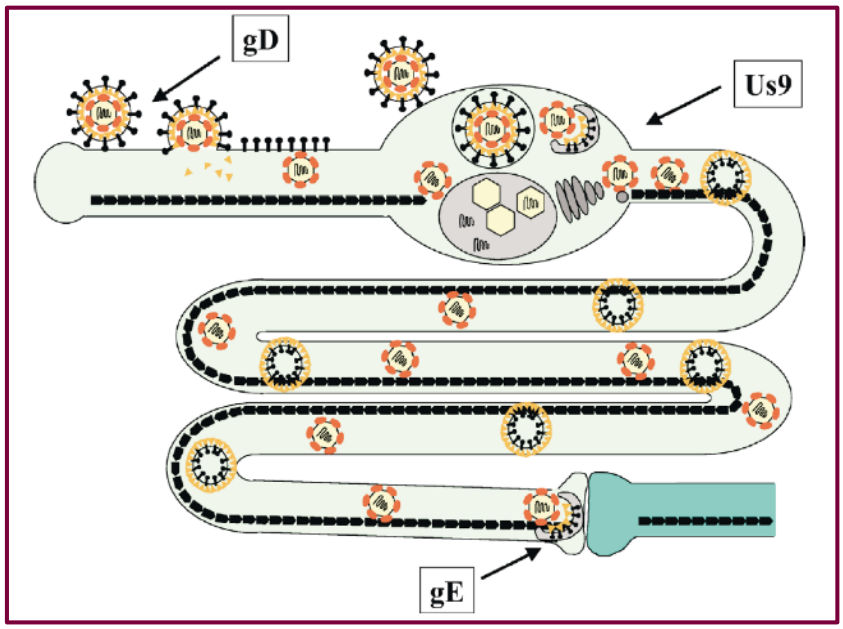

Figure 3: Summary of the possible events that can take place after an α-herpesvirus enters a neuron. gD is important for the virus to be recognized by the host cell upon initial infection. Us9 is responsible for determining which pathway the viral particle will follow after transcription. gE is essential for triggering virion assembly at the axon terminus and synaptic transfer (Mettenleiter, 2003).

which path the virus will take (Mettenleiter, 2003; Tomishima, et al., 2001). Along with synaptic transfer of α-herpesvirus from a neuron, direct cell-to-cell spread to other cells in close proximity such as astrocytes and microglia is possible (Mettenleiter, 2003). This spurious spread greatly reduces the reliability of α-herpesvirus as a transneural tracer.

USE AS A TRANSNEURAL TRACER

There are many unique characteristics of α-herpesvirus that make it a revolutionary transneural tracer. One of the biggest advantages being its capability for bi-directional motion along the axon. Depending on the purpose of the study, a specific direction of travel can be selected for based on the strain of α-herpesvirus (Babic, et al, 1996). This selection is possible due to certain mutations of the gE protein found in the different strains that greatly increases the probability of travel in a certain direction (Ugolini, 2010). For example, attenuated Bartha PrV and McIntyre-B strain of HSV1 prefer to travel in the retrograde direction, whereas, H129 strain of HSV1 prefers to travel in anterograde direction (Hoover and Strick, 1993; Kelly and Strick, 1993 and Zemanick, Strick and Dix, 1991). Additionally, due to α-herpesvirus' ability to enter the PNS it is the viral transneural tracer of choice for studying autonomic innervation, such as those related to heart rate and breathing (Ugolini, 2010).

Despite all of these advantages, there are some crucial limitations of using α-herpesvirus as a transneural tracer. In a natural infection, α-herpesvirus would establish latency in neurons and not undergo active transcription, results in rapid neural degradation of the infected neurons and glial cells. This is caused by a large inflammatory response in the neurons, triggered by the break down of host mRNA, since the virus has not adapted to control the immune response in neurons like it has in epithelial cells (Laurent, Madjar and Greco, 1998 and Smith, Malik and Clements, 2005). These genes are crucial for viral replication; therefore, they cannot be deleted or manipulated to potentially reduce the inflammatory response (Ugolini, 2010). When using α-herpesvirus as a transneural tracer, it is important to design the study in a way that helps mitigate the limitations.

STUDYING OREXIN-CONTAINING NEURONS USING DUAL TRACING WITH ISOGENIC STRAINS OF α-HERPESVIRUS

A prime example of the benefits of using α-herpesvirus as a transneural tracer is seen in a study by Geerling, et al. (2003). The purpose of their study was to determine if orexin-containing neurons of the lateral hypothalamic region of the brain are connected to multiple systems controlled by the sympathetic nervous system, referred to as outflow systems, in rats. Orexin is a neuropeptide that has been shown to have regulatory properties in wakefulness and arousal. The main effect this study focused on was the flight or fight response, specifically, looking at the adrenal gland innervation and the heart, through the stellate ganglion. The authors attempted to elucidate the function and organization of these neurons was a double-tracing method with Barth PrV (Geerling, et al., 2003). This method involves using two isogenic strains of the virus, each tagged with a different protein for visualization. Geerling, et al. (2003) tagged one strain with GFP (BPrV-GFP)

and the other strain with β-galactosidase (BPrV-βgal), a protein that can be stained for easy visualization. Bartha PrV, a retrograde tracer, was selected for this study as they wanted to trace the neural pathway starting from the outflow system to the brain. In this situation, retrograde refers to the direction of travel towards the brain and anterograde would refer to motion away from the brain.

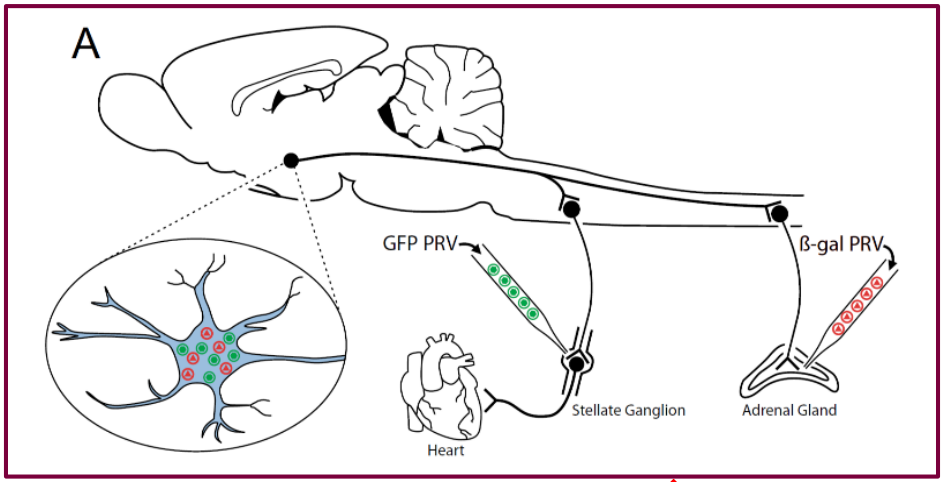

Figure 4: Design of the double tracing study. The GFP tagged strain of pseudorabies virus was injected into the stellate ganglion and the β-gal tagged strain was injected into the adrenal gland. The orexin neurons in the lateral hypothalamic region of the brain were then imaged to see if any contained the colours of both viral strains (Geerling, et al., 2003).

The BPrV-GFP strain was injected into one outflow system, the stellate ganglion. The BPrV-βgal strain was injected into the other outflow system, the adrenal glands (Figure 4). The virus was then allowed to replicate and synaptically transfer naturally over a period of four days, giving it more than enough time to travel up to the lateral hypothalamic region (Geerling, et al., 2003). This process all took place in vivo. If a neuron was infected with both strains, it had to have been synaptically connected to both sympathetic outflow systems, thus, have the possibility to control multiple targets. Geerling, et al. (2003) were able to show orexin-containing neurons that were tagged with both GFP and β-galactosidase (Figure 5).

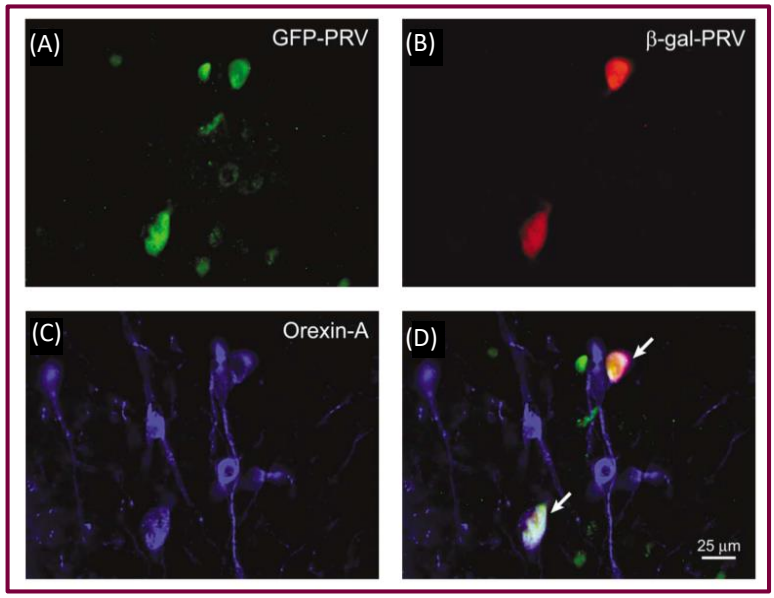

Figure 5: Images of the labelled orexin neurons in the lateral hypothalamic region after PrV was allowed time to replicate and transfer from the initial injection sites. (A) shows all neurons in the region that were infected with BPrV-GFP. (B) shows all neurons in the region that were infected with BPrV-βgal. (C) shows an image of the lateral hypothalamic region with a stain applied to show all orexin-containing neurons. (D) is an overlay of all the previous images. The white arrows indicate the neurons that exhibit colours from both viral strains (Geerling, et al., 2003).

Viral transneural tracing with BPrV was an essential component to the method of this study. The unique characteristics of the α-herpesvirus to enter the CNS from the PNS enabled the study of orexin-containing neurons in the lateral hypothalamic region of the brain. Additionally, the self-amplifying ability of the virus allowed it to cross multiple synapses while remaining at high enough concentrations to be visualized.

RABIES VIRUS

With the development of rabies as a retrograde transneural tracer, it quickly replaced the use of retrograde strains of α-herpesvirus (Ugolini, 2010). However, H129 remains the best option for studies requiring anterograde travel along the axon. Rabies is the only completely reliable and entirely specific transneural tracer currently available, based on the requirements previously discussed. Rabies causes no degradation of infected, meaning there are no restraints on the length of the study. Additionally, rabies can only be synaptically transferred and is not able to undergo direct cell-cell transfer, thus there is no risk of spurious spread (Ugolini, 2010). There are two strains of rabies available, the street strain and

the fixed strain. The fixed strain is approximately 100-10000 times less infectious than the street strain, thus in the context of viral neural tracing only the fixed strain is considered (Dietzschold, Schnell and Koprowski, 2005). Of the fixed strain, the CVS-11 subtype is most commonly used (Ugolini, 2010).

Rabies is able to infect and enter the CNS in all mammals, including primates, from any intramuscular injection site. The one major limitation of rabies is that it can only transfer to the CNS from motor neurons (Tang, et al., 2005). Similar to α-herpesvirus, rabies is a well characterized virus making it easy to determine and control all the necessary factors for successful transneural tracing, such as the required dose. Rabies replicates and travels through neurons in a time dependant manner, allowing the number of synaptic connections to be calculated using the kinetics of propagation of the virus, similarly to α-herpesvirus (Taber, et al., 2005). Rabies can also be easily visualized using immunolabeling with florescent proteins (Taber, et al., 2005).

VIRAL CHARACTERISTICS

Rabies is a small, non-segmented, negative stranded RNA virus with a characteristic bullet shape (Figure 6). Its genome contains up to ten genes, with only five being common between all strains of rabies virus (Schnell, et al., 2010). The natural host species of rabies is bats, however it has a large susceptible host range including insects, fish, mammals, reptiles, and crustaceans (Albertini, et al., 2012). Rabies enters the myocytes by receptor mediated endocytosis (Schnell, et al., 2010). It then quickly moves from myocytes into motor neurons at neuromuscular junctions and then on to the CNS. Once in the neuron the virus will travel to the cell body where genome replication and translation of viral proteins takes places (Schnell, et al., 2010). No

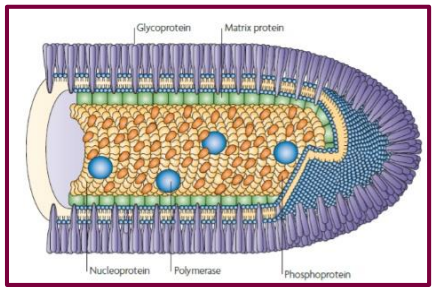

Figure 6: Rabies virus structure. All rabies viruses have a similar structure containing a neucleocapsid, envelope, and membrane glycoproteins (Schnell, et al., 2010).

host cell functions are manipulated or blocked with an initial rabies infection (Ugolini, 2010). These characteristics give rabies its uniquely long asymptomatic incubation period that ranges from 3 weeks to 3 months (Plotkin, 2000).

ENTRY INTO NEURONS

Rabies enters a neuron through receptor-mediated endocytosis, after recognition of its membrane glycoprotein G (Figure 7). While it is still uncertain which specific receptor binds to rabies, neural cell adhesion molecule (NCAM) is believed to play a role in its entry (Schnell, et al., 2010). Afterwards, one of two things are hypothesized to occur (Figure 8). First, the whole virion is transported along the axon in the vesicle from when it was endocytosed. Alternatively, membrane fusion of the virion and the endosome occurs soon after entry into the neuron and then the neucleocapsid is transported alone. Membrane fusion of the virus to the endosome is triggered by the low pH inside the vesicle. Current research more strongly supports the first theory (Albertini, et al., 2012).

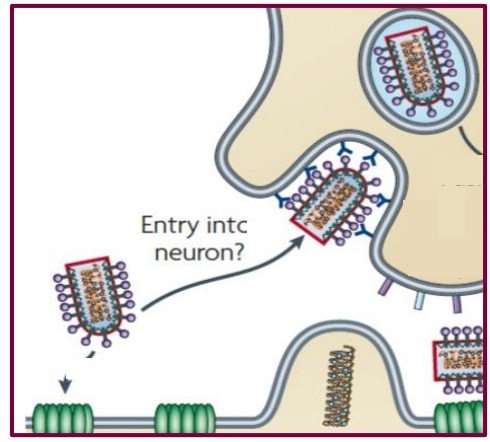

Figure 7: Rabies virus glycoprotein is recognized by the host cell and then enters by receptor mediated endocytosis (Schnell, et al., 2010).

Regardless of the method of transportation, the virus will travel in a retrograde motion, moving along the microtubules in the axon using the host's cytoplasmic dynein motor complex (Albertini, et al., 2012). Rabies can only travel in a retrograde motion, it is not capable of travelling in an anterograde direction along an axon. Once at the cell body, transcription and translation of the viral proteins begins. Very little is known about what

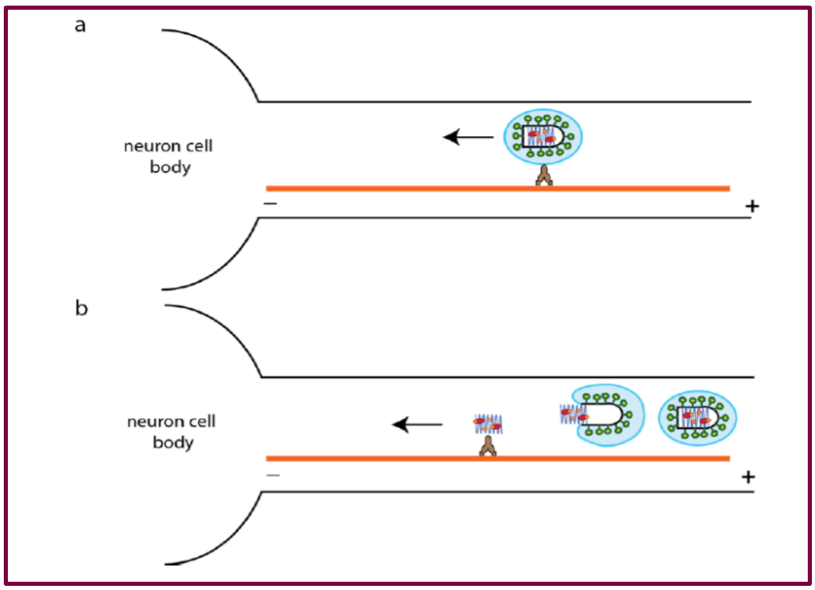

Figure 8: Two possible methods of axonal transport for rabies. (A) shows the whole virion moving along the axon inside a vesicle. (B) shows the early membrane fusion and the neucleocapsid moving along the axon on its own. Research more strongly favours the first method of transport (Albertini, et al., 2012).

triggers primary transcription (Albertini, et al., 2012). After replication, the virus is assembled and transported to the axon terminus. Unlike most neurotrophic viruses, rabies does not use apoptosis as a mechanism to spread in the body. Instead the mature virions will bud from the neuron and undergo synaptic transfer (Schnell, et al., 2010).

USE AS A TRANSNEURAL TRACER

The unique characteristics of rabies make it the most reliable transneural tracer currently available. This is due to the fact that it can only be transferred synaptically, with no spurious spread. There is also no neural degradation as a result of viral infection. Rabies does not induce apoptosis and has evolved to actively replicate in neurons and thus is able to control their immune response. The advantage of using the fixed strain is that it has been attenuated to the point where its incubation period and virulence are stabilized (Schnell, et al., 2010). The incubation period of the fixed strain is approximately one week. In that time period the virus can cross approximately seven synapses, which is more than enough transfers for a successful study (Ugolini, 2010).

There are a few limitations when using rabies as a transneural tracer. Since there is no neuronal degradation caused by infection, it can be difficult to determine the initial infection site. To overcome this limitation, dual tracer techniques can be used with a conventional tracer (Conti, Superti and Tsiang, 1986). The conventional tracer is only capable of spreading to first order neurons from the infection site, thus giving a more precise marker of the initial infection site. Cholera toxin B fragment, in a low concentration, is the only conventional tracer determined to effectively mark the infection site while not effecting the uptake of the rabies virus by the cells (Ugolini, 2010). The other limitation with rabies is the potential for some cytopathic changes (structural changes to the host cell due to a viral infection) in CNS neurons, however these changes are usually negligible (Jackson, 2002).

STUDYING A MODEL OF SCHIZOPHRENIA USING RABIES VIRUS

Technique wise, a notable example of using rabies as a transneural tracer is with a study by Brennand, et al. (2011). The aim of their study was to elucidate cellular and molecular defects caused by schizophrenia, specifically looking at neural connectivity. Schizophrenia (SCZD) is a debilitating neurological disease that affects approximately 1% of the world's population (Sullivan, Kendler and Neale, 2003). Despite its high prevalence, very little is known about the disease due to the difficulty of studying functional neurons in vivo. In order to overcome this problem, Brennand, et al. (2011) created schizophrenia human induced pluripotent stem cells (SCZD iPSC). iPSCs are adult cells that have been genetically reprogrammed to exhibit embryonic stem cell properties by overexpressing the genes and transcription factors that define the embryonic stem cell state (National Institutes of Health, 2015). Embryonic stem cells have the ability to differentiate into any cell of the body. Therefore, they can be directed to differentiate into neurons by expressing the correct mixture of signals and transcription factors normally

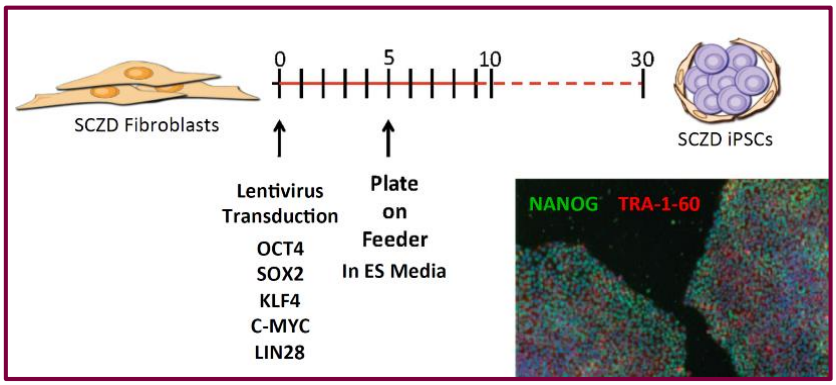

Figure 9: Design of the procedure for creating schizophrenia induced pluripotent stem cells (SCZD iPSC). The fibroblasts were collected from the schizophrenic patient then transduced with transcription factors that define the embryonic stem cell state. The cells were then cultured in a specific media and allowed to populate for 30 days (Brennand, et al., 2011).

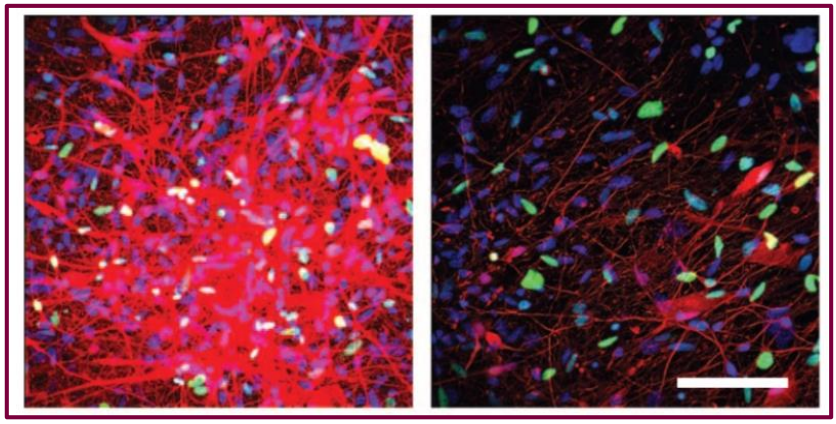

Figure 10: Images of the schizophrenia model after the rabies virus was allowed time to replicate and synaptically transfer. (A) is from an individual without schizophrenia. It shows a high concentration of red neurons indicating synaptic connection. (B) is from an individual with schizophrenia. It shows a low concentration of red neurons and more green neurons indicating very little synaptic connection (Brennand, et al., 2011).

expressed during neural development (National Institutes of Health, 2015).

Brennand, et al. (2011) took samples of and reprogrammed fibroblast cells, the most common cell found in connective tissues such as collagen, from schizophrenic patients. These reprogrammed cells were then cultured and later directed to differentiate into neurons (Figure 9). At the end of this process, Brennand, et al. (2011) had an accessible sample of functional neurons from a schizophrenic patient. Essentially, they created a model of the disease.

With their model they assayed for neural connectivity using rabies as a transneural tracer. First the primary neurons were transduced with GFP using a lentivirus. The rabies virus was then tagged with a RFP and injected into the model one week later (Brennand, et al., 2011). Primary infected cells were both GFP and RFP positive, while cells synaptically connected to the primary cells were GFP negative and RFP positive (Brennand, et al., 2011). Since rabies can only be transferred through synapses, this set up gave a clear indication of neural connectivity by comparing the number of GFP tagged neurons to RFP tagged neurons. After injecting rabies into the model, the virus was allowed to naturally replicate and propagate for 10 days before imaging the sample (Brennand, et al., 2011). This same procedure was then repeated for fibroblast cells from an individual without SCZD as a control.

After examining the images of both samples, Brennand, et al. (2011) were able to show that neurons in the SCZD model had significantly decreased connectivity than the control model (Figure 10). The complete reliability of rabies enabled them to quantify their results using the ratio of GFP tagged neurons to RFP tagged neurons. Additionally, they were able to test multiple antipsychotic drugs on the model and get a measurable result to determine if they had any significant effects on the neural connectivity. Brennand, et al. (2011) exposed both the control and SCZD models to five major antipsychotic drugs: loxapine, clozapine, olanzapine, risperidone, and thioridazine. Only loxapine significantly improved the neural connectivity (Figure 11).

This study would not have been successful before the development of rabies as a transneural tracer. The unique characteristic of rabies to be only synaptically transferred ensured the results were reliable and allowed for some cellular defects linked to SCZD to begin to be defined. Additionally, the use of a virus enabled the study of functional neurons, thus giving a more accurate understanding of the disease.

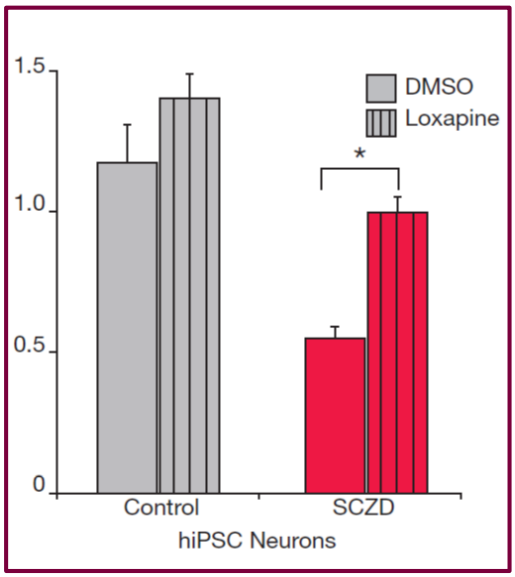

Figure 11: Graph showing the results of treating both control and schizophrenia models with loxapine. The vertical axis represents the ratio of RFP tagged neurons to GFP tagged neurons in each of the images. The solid coloured bar represents the negative control, treated with a solvent. The stripped bars represent the models treated with loxapine. The left, gray bars represent the control model. The right, red bars represent the schizophrenia model and show the significant improvement to neural connectivity after treatment. The error bars are standard error (Brennand, et al., 2011).

FUTURE OUTLOOK

GENETICALLY MODIFIED VIRAL TRANSNEURAL TRACERS

One of the most obvious advancements that could be made with viral transneural tracing is dual tracing with two different viruses in order to study broader systems and connectivity. However, infection of a cell with one virus interferes with the ability of another virus to infect the cell. The efficacy of the second virus to infect the cell exponentially decreases with time. The second virus must infect the previously infected cell within a short period of time, usually within an hour, of the first virus to have a chance for successful replication. This strict time dependant procedure also depends on the dose of each desired viral transneural tracer, making it difficult and tedious to perform. Therefore, researchers are focussed on discovering other more efficient areas of advancement.

The development of reverse genetics gave the ability to genetically manipulate viruses directly. Therefore, this enables viruses to be modified to fit a specific purpose or study and gives the potential for more accurate results while avoiding dual tracing with two different viruses (Callaway, 2008). α-herpesvirus has a large genome with many non-essential genes providing great possibility for genetic manipulation. Rabies has a small genome of only 10 genes at maximum, thus has less opportunity for manipulation. One of the main advantages of using genetically manipulated viruses is the ability to restrict the progression of the virus to a certain neural pathway starting from a specific cell.

Typically, after injecting a viral transneural tracer into a sample it will infect the cells non-specifically and spread through all of the connected synapses. A virus is not potent enough to have successful replication and tracing when injected into only a single target cell (Wickersham et al., 2007). This provides a great limitation to the specificity of studies especially considering similar neighbouring neurons can have drastically different connectivity and functions from each other. To overcome this problem, Wickersham, et al. (2007) designed a study using rabies virus that limited its infectivity

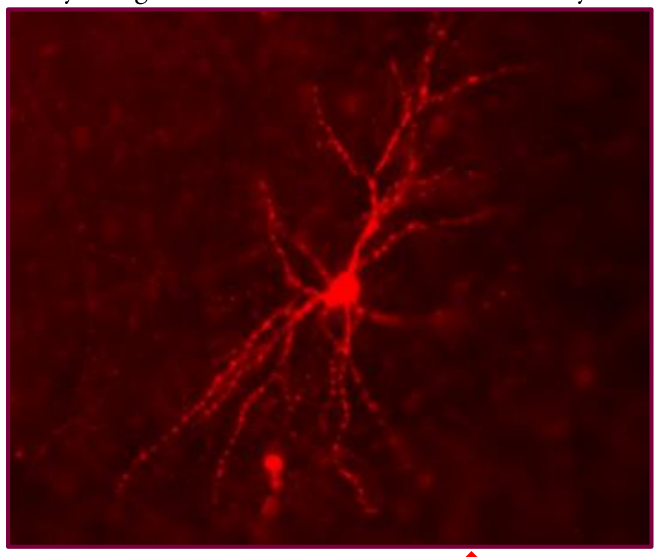

Figure 12: Image of a single stained neuron and its synaptic connections. A genetically modified rabies virus expressing envelope protein A and tagged with RFP was injected into a slice of brain from a rat and allowed to replicate. The target cell was transfected with sub-group A avian sarcoma receptor. The image was captured six days after initial infection showing infection of only the target neuron (Wickersham et al., 2007).

to a specific cell. The G protein, which is the part of the virus recognized by the cell for entry, was deleted. They then expressed the envelope protein A of the avian leukosis virus, which has the unique property of only being recognized by sub-group A avian sarcoma receptor, in the deletion mutant rabies virus (Wickersham, et al., 2007). The gene for the receptor was transfected into the target cell for their study. Therefore, the modified rabies virus would only be able to infect the cell containing the transfected avian receptor. With no other cells expressing the sub-group A avian sarcoma receptor, the rabies virus would be restricted to transsynaptic transfer after replication, therefore unambiguously tracing a specific neural pathway. Wickersham, et al. (2007) tested this procedure in a control experiment in vitro using rat brain slices to show proper infection selectivity. The images of their results showed that after injection of the genetically modified rabies virus into the sample rat brain slice, the virus successfully only infected the target cell (Figure 12).

Theoretically, another possibility for advancement is genetically modifying viruses in order to link connectivity to function in a single study. One of the main goals of neuroscience is to understand how the structure and function of neural circuits relate. Rabies virus recombinants could be designed to express neural activity indicators or light-sensitive ion channels (Ugolini, 2010). This would allow for the monitoring or manipulation of neural activity in vivo, which would be a major step forward in accomplishing the goal of fully understanding neural circuits. The use of rabies as a transneural tracer was essential to enable this emerging technique as it does not cause neural degradation in infected cells (Ugolini, 2010). The use of reverse genetics on viruses is in the early stages, however there is a great possibility that this research will lead to the next major step forward with understanding neural structure and function.

ACKNOWLEDGEMENTS

I would like to thank Dr. Sarah Gretton from the Natural Sciences programs at the University of Leicester for her supervision and guidance in completing this independent study.

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