A new anterograde trans-synaptic tracer based on Sindbis virus

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Abstract

Mapping neural circuits is critical for understanding the structure and function of the nervous system. Engineered viruses are a valuable tool for tracing neural circuits. However, current tracers do not fully meet the needs for this approach because of various drawbacks, such as toxicity and characteristics that are difficult to modify. Therefore, there is an urgent need to develop a new tracer with low toxicity and that allows for long-term studies. In this study, we constructed an engineered Sindbis virus (SINV) expressing enhanced green fluorescent protein (EGFP) reporter gene (SINV-EGFP) and found that it had no significant difference in biological characterization compared with the wild-type Sindbis virus in BHK-21 cells and neurons in vitro. We injected the virus into the visual circuit of mouse brain and found that it infected neurons in the local injected site and anterogradely spread in the neural circuits. Although the efficiency of transmission was limited, the findings demonstrate that SINV can be used as a new anterograde tracer to map neural circuits in mouse brain and that it spreads exclusively in the anterograde direction. Further, use of SINV in mouse brain research will provide longer time windows for circuit tracing than is possible with herpes simplex virus and vesicular stomatitis virus tracers.

Key Words: anterograde; lateral geniculate nucleus; mouse brains; neural circuit; neurons; retina; Sindbis virus; superior colliculus; synapse; tracer

Introduction

Many-branched dendrites and axon fibers form complex networks within the brain. Various neurotropic viruses have been engineered to express reporter genes to trace these neural circuits (Wickersham et al., 2007b; Lo and Anderson, 2011; Zingg et al., 2017). Viral tracers transmit between connected neurons and fluorescent protein is used to visualize the networks. Among these tracers, pseudorabies virus and rabies virus belong to the retrograde tracers. Rabies virus is usually used as a mono-transsynaptic tracer (Kelly and Strick, 2000; Wickersham et al., 2007a) and pseudorabies virus is usually used as a multi-transsynaptic tracer (Jia et al., 2019; Sun et al., 2019). Nearly all adeno-associated viruses (AAVs) are non-transmittable viral tracers that express reporter genes only in the initially injected neurons. AAV vectors label neurons and then transmit to axons, which can provide insight on neural innervation, but are not used for trans-synaptic transmission. Although AAV1 and AAV9 exhibit anterograde trans-synaptic properties with super-high titers, they require cre-dependent transgene expression for amplification (Zingg et al., 2017). The herpes simplex virus type 1 (HSV-1) and vesicular stomatitis virus (VSV) can anterogradely spread within neural circuits (Lo and Anderson, 2011; Harouasaki and Wertz, 2012; Lin et al., 2020; Su et al., 2020).

HSV-129 is a multi-transsynaptic tracer, and H229-ΔTk-tdT can be used as a mono-transsynaptic tracer with AAVs expressing the thymidine kinase gene. However, a major drawback of HSV-1 is terminal absorption; axon terminals of neurons pick up the virus and retrogradely transport it to the soma, which limits the virus’s use for tracing circuits (Diefenbach et al., 2008). Further, HSV-1 has cytotoxicity and animal toxicity. It normally kills experimental mice in 3–7 days, and brain slices of the animals exhibit severe inflammation and tissue damage in the injected sites. In addition, because of the large genome of HSV-1, it is difficult to engineer and manipulate. VSV also has high cytotoxicity and animal toxicity and can lead to the rapid death of mice, which limits tracing to short periods (Li et al., 2019). These virus-based tracers provide powerful tools and methods for neuroscientists. However, the current drawbacks, such as toxicity, limit their use for labeling neural circuits. Therefore, novel tracers are needed.

Sindbis virus (SINV) is a small, enveloped positive-strand RNA virus that belongs to the alphavirus family (Carrasco et al., 2018). SINV transmits between vertebrates, including birds and mammals, in nature by mosquito bites (Straus and Strauss, 1994; Ziegler et al., 2019). Previous studies have shown that SINV prefers to infect neurons rather than glial cells (Furuta et al., 2011; Ziegler et al., 2019). Previous studies have shown that SINV infects neurons rather than glial cells (Furuta et al., 2011; Ziegler et al., 2019).
Figure 2E). At 48 hpi, positive cells were detected in LP, which corresponded to the microcircuit of mouse brain. Therefore, we analyzed the characteristics of SINV and its spread by mapping the neural circuits of mouse brain.

Materials and Methods

Animals
All procedures were approved by Animal Care and Use Committees at Innovation Academy for Precision Measurement Science and Technology, the Chinese Academy of Sciences (approval No. APM2005S30) in 2021. Fetal C57BL/6 mice (for primary cultured neurons) and adult C57BL/6 mice (for viral infection) were used. Adult C57BL/6 mice at 8-week-old, 20–25 g in vivo, and 6-week-old, 20–25 g in vivo, were purchased from an in vivo animal breeding center in Hubei, China. Since SINV infection in vivo infection can be observed until 48 hpi, all animals were sacrificed and detected by EGFP signal at 48 hpi. To confirm the spread of SINV, all animals were used at 48 hpi.

Construction of plasmids
The SINV backbone was from the hybridT12 strain, the nonstructural protein and capsid were from the Toto1101 strain and the structural protein from the Toto1101 strain. The genome of SINV can be easily engineered, including its spread in mapping the neural circuits of mouse brain. Therefore, we analyzed the characteristics of SINV and its spread by mapping the neural circuits of mouse brain.

Cells and viruses
All experiments were performed in a Biosafety Level 2 laboratory and animal facility. Baby hamster kidney cells (BHK-21; American Type Culture Collection, Manassas, VA, USA; ATCC: CVCL_1915) were cultured in Dulbecco’s minimum essential medium (Shanghai BasalMedia Technologies Co., Ltd., Shanghai, China) containing 10% fetal bovine serum (Thermo Fisher, Waltham, MA, USA) and inoculated at 37°C in 5% CO₂. The plasmid was transfected with lipofectamine 2000 reagent (Thermo Fisher) using opti-MEM (Thermo Fisher). After 6 h, the supernatant was discarded and replaced with Dulbecco’s minimum essential medium containing 2% fetal bovine serum at 37°C in 5% CO₂. Then, the supernatant was collected for 24, 36, and 48 h post-transfection (hpt) by plaque assay (Jia et al., 2016) in BHK-21 cells. The primary culture neurons were collected from fetal C57BL/6 mice brains 3 days before delivery. The pregnant mice were anesthetized by pentobarbital sodium (50 mg/kg; Sigma-Aldrich, St. Louis, MO, USA). The fetuses were removed, and then the brain was dissected. The brains were then sectioned using cryostat (Figure 1C). Animals

Virus rescue and biological characterization
In vitro
To characterize the time course of SINV spread in mouse brain, we chose the full-length SINV genome, which contained the EGFP reporter gene under the control of the 265 promoter (Figure 1A). In addition, we analyzed the anterograde spread of SINV-EGFP in vivo (Figure 1B). At 48 hpi, all animals were infected and observed EGFP signal. In addition, the spread of SINV-EGFP in the brain was quantified with the largest percentage of infected cells per group. The percentage of infected cells was analysed by Log-rank test. Graphpad Prism8 (Graphpad Software, San Diego, CA, USA; www.graphpad.com) was used for statistical graphs.

Results

Virus rescue and biological characterization in vitro
The replication-competent vector system contained the full length of the SINV genome, which contained the EGFP reporter gene under the control of the 265 promoter (Figure 1A). At 48 hpi, all animals were infected and observed EGFP signal. In addition, the spread of SINV-EGFP in the brain was quantified with the largest percentage of infected cells per group. The percentage of infected cells was analysed by Log-rank test. Graphpad Prism8 (Graphpad Software, San Diego, CA, USA; www.graphpad.com) was used for statistical graphs.

Virus rescue and biological characterization in vivo
To verify whether the virus infects neurons and spreads within neural circuits in vivo, we chose the full-length SINV genome, which contained the EGFP reporter gene under the control of the 265 promoter (Figure 1A). In addition, we analyzed the anterograde spread of SINV-EGFP in vivo (Figure 1B). At 48 hpi, all animals were infected and observed EGFP signal. In addition, the spread of SINV-EGFP in the brain was quantified with the largest percentage of infected cells per group. The percentage of infected cells was analysed by Log-rank test. Graphpad Prism8 (Graphpad Software, San Diego, CA, USA; www.graphpad.com) was used for statistical graphs.

Virus injection
Animals in the in vivo study are listed in Additional Table 1. Adult C57BL/6 mice (n = 28) were used, and the injection process was performed according to a previous study (Jia et al., 2016). Briefly, 2 µl of SINV-EGFP (3 × 10⁶ PFU/mL) was injected into the vitreous body of the eye, and 150 µl of SINV-EGFP (3 × 10⁶ PFU/mL) was intracerebrally injected into the superior colliculus (SC) and the lateral geniculate nucleus (LGN). The stereotaxic coordinates for the SC were: anterior-posterior, -4.00 mm; medio-lateral, -1.40 mm; and dorso-ventral, -1.80 mm. The stereotaxic coordinates for the LGN were: anterior-posterior, -1.80 mm; medio-lateral, -2.00 mm; and dorso-ventral, -2.50 mm from the Bregma (Paxinos and Franklin, 2013). After 24, 48, 72, and 96 h, the mice were intracranially injected with pentobarbital sodium (50 mg/kg), sacrificed, and fixed in 4% formaldehyde solution overnight. The brain slices were dehydrated in 30% sucrose solution for 48 to 72 h, and sectioned in 40-µm-thick slices using a freezing sectioning machine (CryoStar NX50, Thermo Scientific, San Jose, CA, USA). The brain slices were collected with antifreeze liquid (50% phosphate buffered saline, 20% glycerine, 30% ethylene glycol) for further staining and imaging.

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Figure 1 | The biological characteristics of the SINV-EGFP in vitro.

(A) Diagram of pSINV-EGFP (a) and pSINV-WT (b) genome structures. (B) The kinetics of virus production. Fluorescent images of BHK-21 cells after transfecting pSINV-EGFP and pSINV-WT at different time points. Fluorescence signals could be detected at 12 hpi and increased with time in the pSINV-EGFP group. No fluorescence was detected in the pSINV-WT group. Virus titers at different time points post-transfection were measured by plaque assay. (C) The single-step growth curves of both viruses. These viruses were collected and titrated on BHK-21 cells at different time points post-infection. (D) Plaque sizes of both viruses. The sizes of the viruses were not significantly different. (E) Fluorescent images of cultured primary neurons and BHK-21 cells after infecting with SINV-EGFP. All cells were infected and expressed EGFP. C: Capsid; E3, E2, E1, 6K: structural protein; EGFP: enhanced green fluorescent protein; hpi: hours post-injection; MOI: multiplicity of infection; NSP1–4: nonstructural protein 1–4; UBC: ubiquitin C promoter.

Figure 2 | Injection into the LGN of mice to characterize virus trans-synaptic properties.

(A) Diagram of circuits between retinal ganglion cells, LGN, V1 and V2. Data are expressed as mean ± SD. (B) Positive cells of different brain areas at 96 hours post-infection. (C–E) SINV-EGFP spread transsynaptically in the anterograde direction from injected sites to primary output V1 and secondary output V2 by 96 hpi. A large number of neurons were labeled in the LGN (C), and positive cells were also detected in V1 (D) and V2 (E). The experiments were repeated three times. EGFP: enhanced green fluorescent protein; hpi: hours post-injection; MOI: multiplicity of infection; NSP1–4: nonstructural protein 1–4; UBC: ubiquitin C promoter.

Figure 3 | Injection into the retina of mice to characterize the virus.

We injected 2 μL SINV-EGFP into the vitreous body of the eye of mice, and mice were sacrificed at 96 hpi. (A) Diagram of neural circuit between the RGCs of retina, the LGN and V1. (B) At 96 hpi of subretinal cells, we isolated and expanded retinal cells. RGC axons were labeled by SINV-EGFP. (C, D) Positive signals were detected in the LGN and V1, which indicates that the virus anterogradely spread in the neural circuit. The experiments were repeated three times. EGFP: Enhanced green fluorescent protein; hpi: hours post-injection; LGN: lateral geniculate nucleus; RGCs: retinal ganglion cells; V1: visual cortex area 1.

Figure 4 | The time course of SINV-EGFP spread within the neural circuit.

The superior colliculus (SC) was chosen as the injection site. (A) Diagram of the SC outputs in the neural circuits. (B) The virus was injected into the SC (150 nL, 3 × 10^4 PFU/mL) in adult mice. Three adult mice per group were used for SC injection, and were sacrificed at 24, 48, 72, and 96 hpi. Positive cells were first observed at 24 hpi, and the number of labeled neurons increased with time. In the thalamic nucleus (LP), many positive cells were detected at 48 hpi, and in the LA, positive cells were detected at 96 hpi. (C) The positive cells within each brain area were quantified in every whole-brain slice. Data are expressed as mean ± SD (n = 3 animals for each time point). (D) The lethality after intracranial injection with SINV-EGFP (150 nL, 3 × 10^4 PFU/mL) and VSV-mNeonGreen (150 nL, 2.5 × 10^7 PFU/mL). Survival rate of SINV and VSV infected mice. Two viruses were injected intracranially into the SC of adult mice. The percent survival was analyzed by Log-rank test (n = 5 in each group). EGFP: Enhanced green fluorescent protein; hpi: hours post-injection; LA: lateral amygdala; LP: lateral posterior thalamic nucleus; SC: superior colliculus.

Lethality of SINV-EGFP

To evaluate the lethality of SINV-EGFP, we injected either SINV-EGFP (3 × 10^6 PFU/mL) or VSV-mNeonGreen (2.5 × 10^7 PFU/mL) intracranially into the SC of mice. All of the mice injected with SINV-EGFP survived until day 14 and did not die after an extended period (data not shown), whereas mice injected with VSV-mNeonGreen were dead in 9 to 10 days after injection (Figure 4D). This indicated that SINV-EGFP was not lethal to adult mice.
Discussion

Mapping neural circuits is critical for understanding how the brain works and for the development of treatments for neurological diseases. Although several viral-based tracer systems have been developed to map brain networks, they have some limitations, such as toxicity and non-specific entry sites (Lo and Anderson, 2011; Wojaczynski et al., 2015). For this reason, better viral tracers are needed.

The replication-competent vector system of SINV rapidly replicated and produced high titers of virus after infection in BHK-21 cells. SINV-EGFP reporter gene peak was reached at 36 hpi, and its titer was similar to that of the wild-type. Therefore, SINV-EGFP produced a high titer virus suitable for research without requiring additional concentration methods. This will allow for easy preparation of viral reporter gene systems for other viral-based tracers that usually need to be concentrated for mapping nervous circuits.

Two pathways were selected to determine the direction of SINV-EGFP spread in mouse neural circuits. For the visual circuit, SINV-EGFP was injected into the LGN region and the signals were observed in V1, but no signals were detected in the retina. Next, SINV-EGFP was injected into the retina, and the signals were first observed in the LGN, then signals were detected in V1. These findings indicate that SINV-EGFP spreads in the same anterograde direction in mouse neural circuits. For the collicular-thalamic-amygdala circuit, SINV-EGFP was injected into the SC, and the signals were first observed in the LP, and then observed in the LA. These findings further support that SINV-EGFP anterogradely spreads in mouse neural circuits.

In an analysis of the lethality of SINV-EGFP, all of the virus-infected animals survived for well beyond our 14-day time point. In contrast, other anterograde viral tracers, such as VSV and HSV-1, have shown shorter windows for tracing experimentally (Liu et al., 2004; Belabbes et al., 2011; Xu et al., 2020). Thus, SINV-EGFP may provide longer time windows for circuit tracing.

Interestingly, SINV infects and transmits between vertebrates including birds and mammals. SINV was first isolated from feline patients in 1961 (Adouchief et al., 2016). In addition, seroprevalence surveys showed that humans can be infected by SINV from dogs, cats, and birds. SINV-EGFP reporter gene systems were used for mapping nervous circuits. In future research, we will investigate ways to further reduce the cytotoxicity of SINV.

In conclusion, we constructed an SINV-EGFP tracer for mapping nervous circuits of mouse brain. We could easily produce a high-titer of the virus expressing a reporter gene in vitro, and it did not influence the biological characterization of SINV. SINV-EGFP labeled neurons and spread exclusively in the anterograde direction within neural circuits. The efficiency of its trans-synaptic spread was limited and should be improved. SINV was less toxic to adult mice than other anterograde viral tracers and has potential as a useful tracer for neuroscience research in the future.

Acknowledgments: We are grateful to Dr. Jean-Pierre Levraud (Macrophages and Development of Immunity, Institut Pasteur) for his kind gift of SINV vector. Authors H. Xu and X. F. Li provided the viral stock design and experimental analysis; F. Li, experimental implementation: XWS, PL; manuscript draft: XWS, FL. All authors have read and approved the final manuscript.

Conflicts of interest: The authors declare no competing financial interests. Editorial responsibility: Editorial responsibility of this Research Article is with Dr. Patrick Card.

Data availability statement: All data generated during this study are included in this published article and its supplementary information files.

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Additional Table 1: The number of animals used in each experiment.

References

Adouchief S, Smura T, Sane J, Vapaliappan O, Korkesia S (2016) Sindbis virus as a human pathogen: an epidemiological and clinical picture and implications. Rev Med Virol 26:490-513.

Beier KT, Borchg BV, El Dafani RN, Huberman AD, Demb JB, Creek EO (2013) Transsynaptic tracing with vesicular stomatitis virus reveals novel retinotopic organization. J Neurosci 33:53-61.

Beier KT, Čermáková A, Oldenburg M, Wright JN, Lo S, Loh Y, Bailey R, Creek EO (2011) Anterograde or retrograde transsynaptic labeling of CNS neurons with vesicular stomatitis virus vectors. Proc Natl Acad Sci U S A 108:10654-10659.

Bredenbeck P, Frolow I, Rice CM, Schlegel SR (1993) Sindbis virus expression vectors: packaging of RNA replication. J Virol 67:4319-4326.

Burgin R, Bubulya EV, Velardo MJ, Moshier P, Zolotukhin J, Beier P, Mandel R, Muzycka N (2004) Recombinant AAV viral vectors pseudotyped with vesicular stomatitis virus serotype 1 and 2 exhibit differential efficiency of transduction after delivery to different regions of the central nervous system. Mol Ther 10:302-317.

Carrasco I, Sanz MA, González-Ameña E (2018) The regulation of translation in alphavirus-infected cells. Virus Res 250:1-10.

Diefenbach Rz, Miranda-Saikena M, Douglas MW, Cunningham A (2008) Transport and egress of encephalitis viruses in neuroinvasion. J Virol 82:1385-1391.

Ehrengruber MU, Lustig S, Jung S, Ziemann S, Bachmann L, Kalden BK (2011) Sensory maps in the olfactory cortex defined by long range viral tracing of single neurons. Nature 472:217-220.

Hanif, Hahn YS, Braciale TJ, Rice CM (1992) Infectious Sindbis virus transsynaptic expression vectors for studying antigen processing and presentation. Proc Natl Acad Sci U S A 89:2679-2683.

Harauzicko D, Wets G (2012) Second-site mutations selected in trans-synaptic retrograde viral vectors. Nature Methods 9:63-67.

Kurkova Cs, Huchmán E, Uscielweg-Buñag, Ng, Ntouka, Li, Mathé, Hufnagle, Vahabzadeh, Apatlar, Ouzounis. (2008) Sindbis virus infection in resident mouse neurons, birds, and humans. Front. Microbiol Infect Dis 14:47.

L. Li, S. Dow J, Kodono K, Zhi L (2019) Trans-synaptic neural circuit tracing with neurotrophic virus. Nature Neurosci 22:909-920.

Letchy RD, Power AT, Stojdl DF, Bell ICU (2004) Vesicular stomatitis virus: re-inventing the bullet. Trends Mol Med 10:210-216.

Lin K, Zhang X, Ying M, Li, Tao S, Zhu H, Xu, H, Xu (2020) A mutant vesicular stomatitis virus with reduced cytotoxicity and enhanced trans-synaptic efficiency. Mol Brain 13:49.

Lo, Anderson DI (2011) A Ce-dependent, anterograde transsynaptic viral tracer for mapping output pathways of genetically marked neurons. Neuron. 72(2):263-276.

Lundstrom K (2017) Oncolytic alphaviruses in cancer immunotherapy. Vaccines 5:9.

Lustig S, Jackson AC, Hahn ENS, Griffin DE, Strauss EG, Strauss JH (1988) Molecular basis of Sindbis virus neuroinvasiveness in mice. J Virol 62:2329-2336.

Mounce BC, Poirier EZ, Passo G, Simon-Loree E, Ceraro T, Pratt M, Stapleton KA, Moratorio O, Sakan M, Leavis JP, Vignuzzi M (2016) Interferon-induced spermidine-spermine acetylationtransferase and polyamine depletion restrict Zika and Chikungunya viruses. Cell Host Microbe 23:35-50.

Passo G, Langevin C, Palha N, Mounce BC, Brotoli V, Affatato P, De Boe J, Joly JS, Vignuzzi M, Saleh MC, Herbomel P, Boudinot P, Leuvarad P (2017) Imaging of viral neurouvasion in the zebrafish. Neuron 95:360-374.

Lin K, Zhong X, Ying M, Li L, Tao S, Zhu X, Hu, Chen K, Hu, C, Xu F, Wang L (2015) Processing of visually evoked innate fear by a non-canonical thalamic pathway. Nat Commun 6:5765.

Lichty BD, Power AT, Stojdl DF, Bell ICU (2004) Vesicular stomatitis virus: re-inventing the bullet. Trends Mol Med 10:210-216.

Lin K, Zhong X, Ying M, Li L, Tao S, Zhu X, Hu, C, Xu F (2020) A mutant vesicular stomatitis virus with reduced cytotoxicity and enhanced trans-synaptic efficiency. Mol Brain 13:49.

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Lundstrom K (2017) Oncolytic alphaviruses in cancer immunotherapy. Vaccines 5:9.

Lustig S, Jackson AC, Hahn ENS, Griffin DE, Strauss EG, Strauss JH (1988) Molecular basis of Sindbis virus neuroinvasiveness in mice. J Virol 62:2329-2336.

Mounce BC, Poirier EZ, Passo G, Simon-Loree E, Ceraro T, Pratt M, Stapleton KA, Moratorio O, Sakan M, Leavis JP, Vignuzzi M (2016) Interferon-induced spermidine-spermine acetylationtransferase and polyamine depletion restrict Zika and Chikungunya viruses. Cell Host Microbe 23:35-50.

Passo G, Langevin C, Palha N, Mounce BC, Brotoli V, Affatato P, De Boe J, Joly JS, Vignuzzi M, Saleh MC, Herbomel P, Boudinot P, Leuvarad P (2017) Imaging of viral neurouvasion in the zebrafish. Neuron 95:360-374.

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### Additional Table 1 The number of animals used in each experiment

| Injection site                        | Number of animals | Time of sacrificed (h) |
|---------------------------------------|-------------------|------------------------|
| lateral geniculate nucleus            | 3                 | 96                     |
| Retina                                | 3                 | 96                     |
| Superior colliculus (for Figure 4B, C)| 12 (3 animals each group) | 24, 48, 72, 96        |
| Superior colliculus (for Figure 4D)   | 10 (5 animals each group) | According to the time of death |