

Neuronal Tract Tracing in the Rat Trigeminal Sensory System

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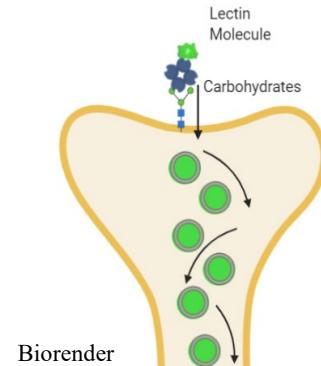
Texas Woman's University

8/6/2020

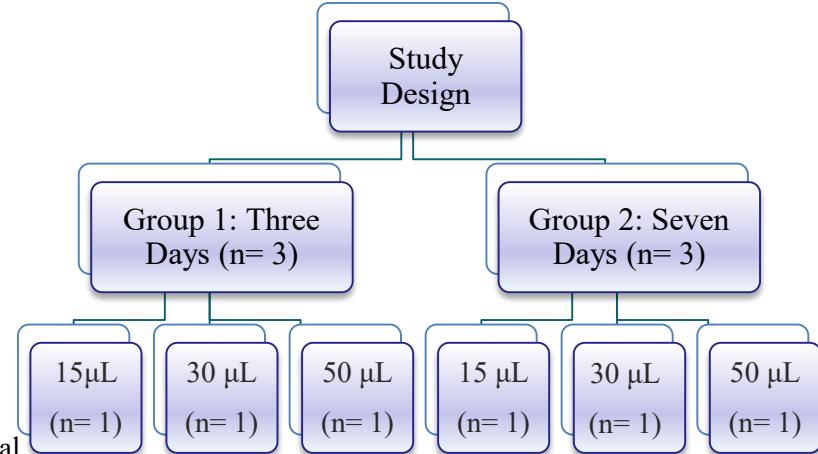
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Introduction

- Neuronal tract tracers are neuroanatomical approaches that allow labeling of neuronal pathways
 - Retrograde (from the nerve ending to cell body)
 - Anterograde (cell body to nerve ending)
 - Trans-synaptic tracer (labels multiple neuronal connections)
- Wheat Germ Agglutinin (WGA) is a common trans-synaptic tracer. The most current form of WGA is the WGA-Alexa-488 trans-synaptic fluorescent dye. Literature has demonstrated successful labeling of some neural pathways using WGA-488; yet, the use of WGA-488 to investigate many other areas remains to be explored (Reeber, Gebre et al. 2011, Buttry and Goshgarian 2014, Buttry and Goshgarian 2015, Levy, White et al. 2017).
- Major challenges: 1) poor visibility and 2) ineffective labeling between the synapses of neurons.



Methodology



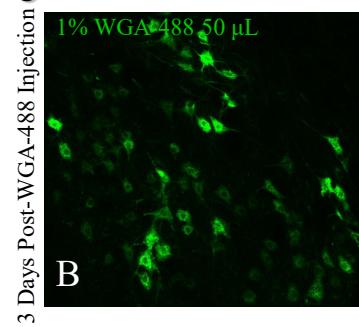
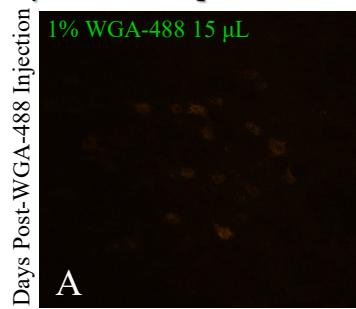
Purpose: 1) To determine the ideal timing and volume of WGA-488 required to label brain regions in the trigeminal system, and 2) to visualize the trigeminal sensory neuron projections from the orofacial region to the Trigeminal Ganglia and Trigeminal Nucleus Caudalis of the Brainstem.

Injection sites:
Left Vibrissal Cheek Pad (VP)
Right Temporomandibular Joint (TMJ)

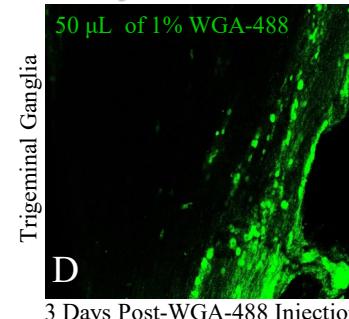
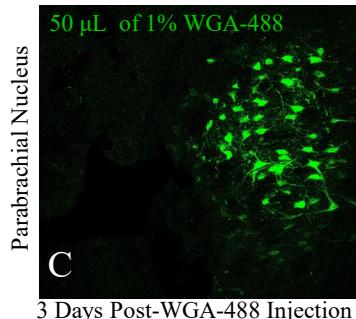
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Results

50 μ L is the Optimal Working Volume for 1% WGA-488



WGA-488+ Neuronal Projections



Numerous Brain Regions Receive WGA-488+
Neuronal Projections

Three Day Tracer	Retrograde Labelling
Spinal trigeminal tract	+++
Periaqueductal Gray	++
Parabrachial nucleus	+++
Medial Lemiscus	+++
Trigeminal Ganglia	+++
Sensory Root of Trigeminal Nerve	+++
Reticular Nucleus	+++

Key

Low Labeling: +

Moderate Labeling: ++

Dense Labeling: +++

Conclusion

We report that the optimal WGA-488 volume is 50 μ L and the time course is 3 days. This study paved the path for deeper analysis of the trigeminal system. Ultimately, we aimed to obtain knowledge on the orofacial pain neuroanatomy and sensory system in male and female rats.

References

Sannajust, S., I. Imbert, V. Eaton, T. Henderson, L. Liaw, M. May, M. F. Barbe and T. King (2019). "Females have greater susceptibility to develop ongoing pain and central sensitization in a rat model of temporomandibular joint pain." *Pain* **160**(9): 2036-2049.

Basbaum, A. I., D. M. Bautista, G. Scherrer and D. Julius (2009). "Cellular and molecular mechanisms of pain." *Cell* **139**(2): 267-284.

Buttry, J. L. and H. G. Goshgarian (2014). "Injection of WGA-Alexa 488 into the ipsilateral hemidiaphragm of acutely and chronically C2 hemisectioned rats reveals activity-dependent synaptic plasticity in the respiratory motor pathways." *Exp Neurol* **261**: 440-450.

Buttry, J. L. and H. G. Goshgarian (2015). "WGA-Alexa transsynaptic labeling in the phrenic motor system of adult rats: Intrapleural injection versus intradiaphragmatic injection." *J Neurosci Methods* **241**: 137-145.

Levy, S. L., J. J. White, E. P. Lackey, L. Schwartz and R. V. Sillitoe (2017). "WGA-Alexa Conjugates for Axonal Tracing." *Curr Protoc Neurosci* **79**: 1 28 21-21 28 24.

Li, J. N. and P. L. Sheets (2018). "The central amygdala to periaqueductal gray pathway comprises intrinsically distinct neurons differentially affected in a model of inflammatory pain." *J Physiol* **596**(24): 6289-6305.

Oztas, D. Y., M. Altunbek, D. Uzunoglu, H. Yilmaz, D. Cetin, Z. Suludere and M. Culha (2019). "Tracing Size and Surface Chemistry-Dependent Endosomal Uptake of Gold Nanoparticles Using Surface-Enhanced Raman Scattering." *Langmuir* **35**(11): 4020-4028.

Reeber, S. L., S. A. Gebre, N. Filatova and R. V. Sillitoe (2011). "Revealing neural circuit topography in multi-color." *J Vis Exp*(57).

Wu, P., D. Arris, M. Grayson, C. N. Hung and S. Ruparel (2018). "Characterization of sensory neuronal subtypes innervating mouse tongue." *PLoS One* **13**(11): e0207069.

Xu, L., H. Jiang, Y. Feng, P. Cao, J. Ke and X. Long (2019). "Peripheral and central substance P expression in rat CFA-induced TMJ synovitis pain." *Mol Pain* **15**: 1744806919866340.

Goshgarian, Harry G. *The Pattern and Extent of Retrograde Transsynaptic Transport of WGA-Alexa 488 in the Phrenic Motor System Is Dependent upon the Site of Application.* NIH Public Access, 2014.

Christine, et al. "A Student's Guide to Neural Circuit Tracing." *Frontiers*, Frontiers, 12 Aug. 2019.

"Lectins and Other Carbohydrate-Binding Proteins-Section 7.7." *Thermo Fisher Scientific – US*.