APPLICATION NOTE

Enhancing Localization of AAV

True Gas-Tight System for Precision Drug Delivery

A 2023 study was conducted using an inhibitory DREADDS (Designer Drugs Exclusively Activated by Designer Receptors) virus carrying the inhibitory DREADDS receptor and florescent reporter (AAV5-hSyn-hM4D(Gi)-mCherry) or a control virus carrying a florescent reporter (AAV5-hSyn-eGFP) to target anterior dorsal thalamic neurons (ADN). The ADN has been shown to play a notable role in driving accurate spatial representations, projecting head direction information to update place cell representations of space via the hippocampus. Proper localization was critical to appropriately assess the relationship between the ADN and the CA1 of the hippocampus during spatial navigation, before, during and after silencing of the thalamic structure.

Using a competitor 10µL syringe with a needle (non-insertable to the syringe barrel body), showed significant inconsistency in delivering AAV vectors across a sample of C57BL/6J mice (n = 29) versus AAV delivery utilizing World Precision Instruments' NanoFil™ Gas-Tight Syringe system (n = 32). Using the competitor syringe, marketed as gas-tight, was found to carry about 10µL of dead volume between its coupling mechanism between the plunger to needle base. The NanoFil™ system was identified as having zero dead volume, where the needle inserts directly into the syringe barrel for a true gas-tight system.

Using the competitor syringe model, the success rate of AAV localization to the ADN was about 28%, where 8 out of 29 subjects showed positive ADN localization. 21 of the total subjects either showed complete leakage of the viral vector, unilateral expression, or total absence of positively tagged neurons. All priming and loading steps remained consistent between use of the competitor syringe model versus the NanoFil™ Gas-Tight Syringe system. Success rate of AAV localization using the NanoFil™ syringe resulted in an 87% success rate of localized vector into the ADN, where 28/32 subjects showed positive localization of AAV to the ADN—a roughly 59% increase in targeting success using the NanoFil™ Gas-Tight system.

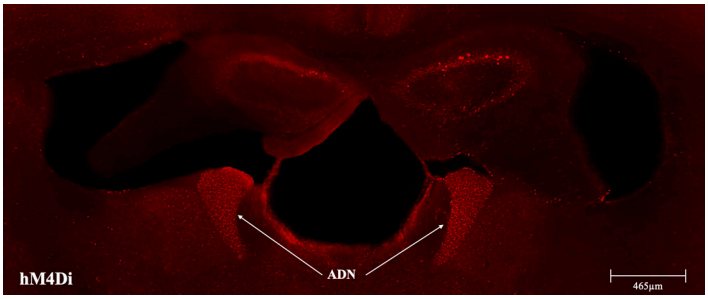

Fig. 1—**Expression of Fluorescent Reporter in Anterior Thalamic Nuclei (ADN): hM4Di Localization.** *Histological verification of bilateral infusion of experimental DREADD virus AAV5-hSyn-hM4D(Gi)-mCherry placement into the anterior thalamic nuclei using a NanoFil 10µL syringe (World Precision Instruments, LLC). Arrowheads indicate the bi-lateral localization of the fluorescent tag in the anterior dorsal region of the thalamus (-2.50mm V). 50µm slice, -1.01mm from bregma.*

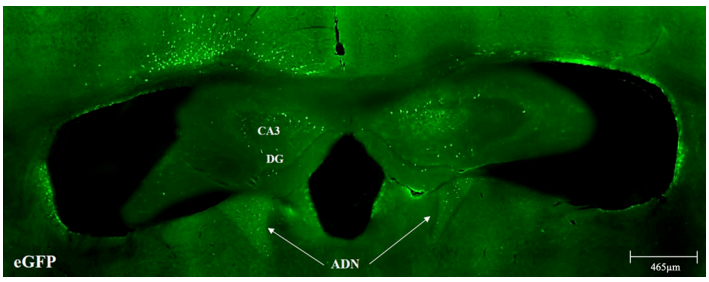

Fig. 2—**Expression of Fluorescent Reporter in Anterior Thalamic Nuclei (ADN): eGFP Localization**. *Histological verification of bilateral infusion of control AAV5-hSyn-eGFP placement into the anterior thalamic nuclei using a NanoFil 10µL syringe (World Precision Instruments, LLC). Arrowheads indicate the bi-lateral localization of the fluorescent tag in the anterior dorsal region of the thalamus (-2.50mm V). 50 µm slice, -0.98mm from bregma.*

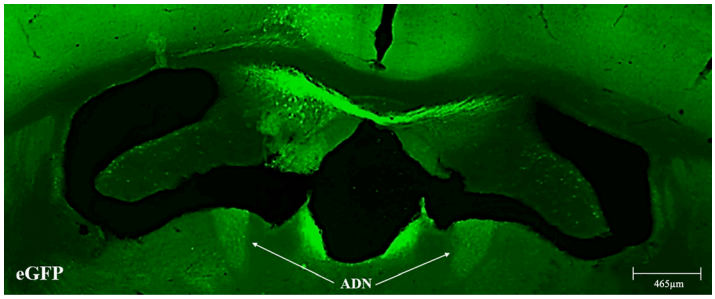

Fig. 3—**Off-Target eGFP Expression of Fluorescent Reporter in Anterior Thalamic Nuclei.** *eGFP subject that was included in the study displaying viral leakage during histological analysis. Occurring in the hippocampal region, leakage of viral vector using a popular competitor gas-tight syringe, with ~10µL dead volume. While anterior dorsal thalamus had been targeted, a majority of control virus had dispensed superiorly into hippocampus. 50 µm; -0.92mm from bregma.*

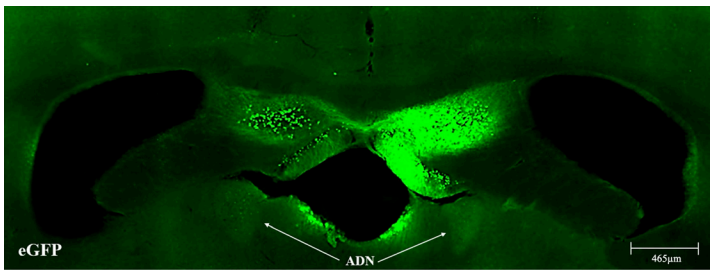

Fig. 4—**Off-Target eGFP Expression of Fluorescent Reporter in Anterior Thalamic Nuclei.** *eGFP subject that was included in the study displaying viral leakage during histological analysis. Occurring in the hippocampal region, leakage of viral vector using a popular competitor gas-tight syringe, with ~10µL dead volume. While anterior dorsal thalamus had been very minimally targeted, a majority of the control virus had dispensed superiorly into hippocampus. 50 µm; -0.94mm from bregma.*

Crafton, B. & Stackman, R. (2023). *Head direction cell network and spatial navigation: effects of silencing anterodorsal thalamic neurons using DREADDS* [Unpublished manuscript]. Charles E. Schmidt College of Science: Department of Psychology, Florida Atlantic University.

Conclusions

Although there was some success using the competitor syringe system, the inconsistency of the performance makes for extended study timelines due to the failure rate of placement, primarily with the compromised gas-tight design. WPI's NanoFil™ Gas-Tight Injection System has a specific design made for a completely gas-tight, vacuum-like environment to be established with its contact of needle-to-plunger. The NanoFil™ needles insert directly into the syringe barrel, making 1:1 contact between the syringe plunger and needle base—creating a virtually *zero dead volume system*. The control and precision of low-volume sample delivery is considerably improved with this design, and permits for accelerated application timelines, as well as decreased failure rates for desired sample localization. While many competitors claim to boast a gas-tight design, it is critical to consider the design of the syringe versus needle coupling mechanism when choosing the best injection system for your application. Try WPI's NanoFil™ Gas-Tight Injection System for results you can trust, time and time again.

Publications

Adenosine Triggers Early Astrocyte Reactivity That Provokes Microglial Responses And Drives The Pathogenesis Of Sepsis-Associated Encephalopathy In Mice

Guo, Q., Gobbo, D., Zhao, N., Zhang, H., Awuku, N.O., Liu, Q., Fang, L.P., Gampfer, T.M., Meyer, M.R., Zhao, R., Bai, X., Bian, S., Scheller, A., Kirchhoff, F., & Huang, W. (2024). Adenosine triggers early astrocyte reactivity that provokes microglial responses and drives the pathogenesis of sepsis-associated encephalopathy in mice. *Nature Communications, 15*; 6340. https://doi.org/10.1038/s41467-024-50466-y

A Bistable Inhibitory Optogpcr For Multiplexed Optogenetic Control Of Neural Circuits

Wietek, J., Nozownik, A., Pulin, M., Saraf-Sinik, I., Matosevich, N., Gowrishankar, R., Gat, A., Malan, D., Brown, B.J., Dine, J., Imambocus, B.N., Levy, R., Sauter, K., Litvin, A., Regev, N., Subramaniam, S., Abrera, K., Summarli, D., Goren, E.M., Mizrachi, G., Bitton, E., Benjamin, A., Copits, B.A., Sasse, P., Rost, B.R., Schmitz, D., Bruchas, M.R., Soba, P., Oren-Suissa, M., Nir, Y., Wiegert, J.S., & Yizhar, O. (2024). A bistable inhibitory optoGPCR for multiplexed optogenetic control of neural circuits. *Nature Methods, 21*; pg. 1275–1287. https://doi.org/10.1038/s41592-024-02285-8

Delivery Of Cdnf By Aav-Mediated Gene Transfer Protects Dopamine Neurons And Regulates Er Stress And Inflammation In An Acute Mptp Mouse Model Of Parkinson's Disease

Nam, J., Richie, C.T., Harvey, B.K., & Voutilainen, M.H. (2024). Delivery of CDNF byAAV-mediated gene transfer protects dopamine neurons and regulates ER stress and inflammation in an acute MPTP mouse model of Parkinson's disease. *Communications Biology, 7*; 966. https://pubmed.ncbi.nlm.nih.gov/39019902/

Chronic Stress Deficits In Reward Behaviour Co-Occur With Low Nucleus Accumbens Dopamine Activity During Reward Anticipation Specifically

Zhang, C., Dulinskas, R., Ineichen, C., Greter, A., Sigrist, H., Li, Y., Alanis-Lobato, G., Hengerer, B., & Pryce, C.R. (2024). Chronic stress deficits in reward behaviour co-occur with low nucleus accumbens dopamine activity during reward anticipation specifically. *Communications Biology, 7*; 966. https://doi.org/10.1038/s42003-024-06658-9

An Adeno-Associated Virus Variant Enabling Efficient Ocular-Directed Gene Delivery Across Species

Luo, S., Jiang, H., Li, Q., Qin, Y., Yang, S., Li, J., Xu, L., Gou, Y., Zhang, Y., Liu, F., Ke, X., Zheng, Q., & Sun, X. (2024). An adeno-associated virus variant enabling efficient ocular-directed gene delivery across species. *Nature communications, 15*(1), 3780. https://doi.org/10.1038/s41467-024-48221-4

See the full bibliography

For a comprehensive list of references, please visit the PUBLICATIONS section of our website.

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