THORNE LABORATORY

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B.S.	Chemical Engineering, University of Washington (Seattle), 1990
Ph.D.	Pharmaceutics, University of Minnesota (Minneapolis), 2002
Postdoc	Physiology & Neuroscience, New York University (New York City), 2008

RESEARCH FOCUS: Targeted central nervous system (CNS) drug delivery; transport processes for distribution within the CNS; optimization of vectors for CNS applications

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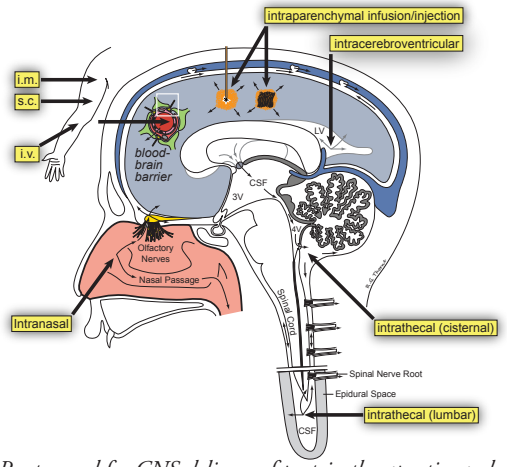
Dr. Thorne received a B.S. in chemical engineering from the University of Washington and a Ph.D. in Pharmaceutics from the University of Minnesota, where he pioneered the study of nasal pathways for CNS delivery with an emphasis on protein tracers and neurotrophic factors in the laboratory of Bill Frey. His postdoctoral work in the laboratory of Charles Nicholson (New York University School of Medicine) focused on studying the diffusion of macromolecules and nanoparticles in brain, providing the first *in vivo* data on protein and nanoparticle diffusion in the CNS as well as identifying several key factors affecting large molecule distribution in this vitally important biological compartment. Dr. Thorne is an active member of both *AAPS* and the *Society for Neuroscience*, a founding member and part of the inuagural steering council for the *International Brain Barriers Society* (www.ibbsoc.org/), and the 2014 Vice Chair-elect (Chair-elect, 2016) for the *'Barriers of the CNS' Gordon Research Conference* (www.grc.org/conferences.aspx?id=0000415), the premier meeting in the field of brain barriers science and CNS drug delivery. His peer-reviewed, published work (11 research papers and 2 review articles) has been cited over 950 times and profiled by *Faculty of 1000 Biology* (www.f1000biology.com/article/id/1019496/evaluation).

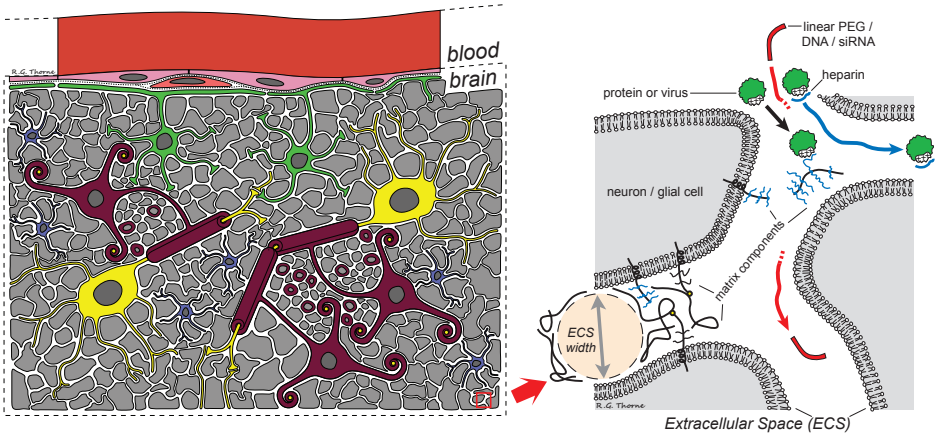
RESEARCH OVERVIEW. *Dr. Thorne's research is currently focused on two primary goals: (i) To identify the factors affecting the diffusion of proteins, genes, viral vectors and nanoparticles inside the developing, adult and pathologic CNS and (ii) To identify the pathways and mechanisms allowing substances to enter the CNS following intranasal administation, a promising alternative route for CNS drug delivery.*

Protein and gene therapies for CNS disorders like Alzheimer's disease, Parkinson's disease and stroke have been limited by two related yet distinct problems. The first concerns the difficulty associated with delivering a protein, gene or drug delivery vector into the CNS across the barriers that separate the blood from brain interstitial and cerebrospinal fluids. The second concerns the uncertainty surrounding what happens on the brain side of these barriers once a substance is able to pass them. Surprisingly, little information exists to predict the distribution of substances following their entry into the CNS. These problems have much to do with our current reliance on small molecule drugs to treat neurological illnesses; indeed, no CNS-acting biopharmaceutical product has yet received approval despite the existence of thousands of exceptionally promising protein and gene therapy candidates. Dr. Thorne's highly translational research addresses these problems using a multidisciplinary approach spanning pharmaceutics, neuroscience, biophysics, engineering and structural biology. Other interests include the use of neurotrophic factors, cytokines, siRNA and other biopharmaceutical products in animal models of disease, the design/optimization of protein conjugates, viral vectors and nanoparticles for CNS applications and blood-brain barrier modification strategies.

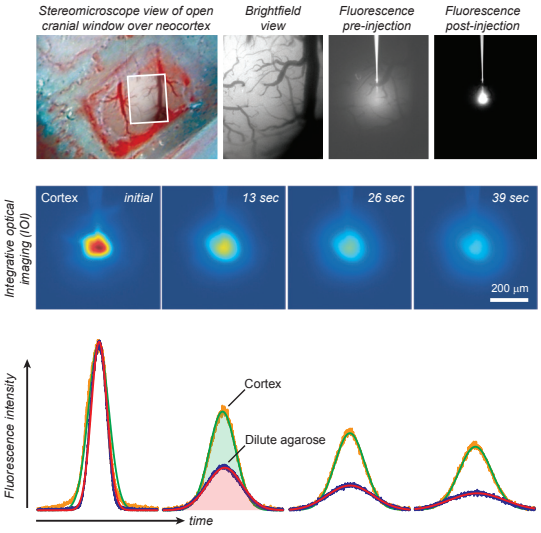


Routes used for CNS delivery of protein therapeutics and gene therapy vectors (from Thorne & Frey, 2001).

PROJECT 1 - DIFFUSION OF DRUGS IN BRAIN TISSUE. All CNS drugs and vectors must navigate the extracellular space (ECS) to exert their effects after crossing the blood-brain barrier or following direct delivery. Optimal CNS drug delivery will require targeted strategies that release substances at specific sites and allow for some degree of local distribution following their release (Thorne & Frey, 2001). Diffusion within the narrow extracellular spaces of the brain is essential for this distribution. We have used integrative optical imaging (IOI), a method for measuring diffusion coefficients of fluorescently labeled substances, as a tool to provide valuable insights into the factors governing diffusion in the brain. While the diffusion properties of specific substances, e.g. growth factors and synthetic



Narrow extracellular spaces between brain cells provide the final transport pathway all drugs must diffuse through to exert their effect after crossing the blood-brain barrier or following direct injection.



Integrative optical imaging (IOI) method for measuring diffusion in dilute agarose (free solution) and in brain ECS. Intensity profiles are fit to provide diffusion coefficients (from Thorne & Nicholson, 2006; Thorne et al, 2008).

polymers such as polyethylene glycol, have obvious importance for their therapeutic application (Thorne et al, 2004 and 2005), diffusion measurements may also be used to probe general aspects of ECS structure and composition that, in turn, can provide critical guidance for the design and optimization of drugs. Accordingly, we have used *in vivo* diffusion measurements to evaluate the width of brain ECS (Thorne & Nicholson, 2006) and the concentration of an important component of the extracellular matrix (ECM), heparan sulfate proteoglycans (Thorne et al, 2008). Our findings suggest ECS width, estimated at 40-60 nm (*normal, adult*), will restrict the movement of many drugs and drug delivery vectors larger than a few nm in size due to steric hindrance and wall drag. Substances that bind heparan sulfate proteoglycans, a prominent component of brain ECM and cell surfaces, will be restricted even further in their movement. More recent studies have begun to explore the diffusion properties of therapeutically important oligonucleotides (siRNA and antisense; Thorne & Nicholson, 2009a, 2009b, 2010). This work employs a variety of experimental methods [*in vitro* (dilute agarose, brain slices) and *in vivo* optical imaging; labeling and characterization of novel fluorescent probes; application of quantitative models; electron microscopy] and is especially relevant for the design and enhancement of transvascular strategies for CNS drug delivery, particularly those using nanoparticles, and the surgical infusion method called convection enhanced delivery (Thorne, 2009).

Wolak, Lochhead & Thorne (2011). *In vivo* optical imaging of antibody distribution following injection into brain. ***AAPS Annual Meeting & Exposition*** (26 Oct 2011; Washington DC).

Thorne & Nicholson (2010). *In vivo* optical imaging of siRNA diffusion in brain extracellular space. ***Society for Neuroscience Annual Meeting*** (14 Nov 2010; San Diego, CA).

Thorne & Nicholson (2009a). *In vivo* optical imaging of oligonucleotide distribution following injection into brain. ***AAPS Annual Meeting & Exposition***, abstract T2266.

Thorne (2009). More factors affecting distribution during and after infusion (http://www.pdonlineresearch.org/responses/3608/180/more-factors-affecting-distribution-during-and-after-infusion).

Thorne & Nicholson (2009b). *In vivo* optical imaging of oligonucleotide diffusion in brain extracellular space: dramatically restricted transport compared to polyethylene glycol. ***SFN***, abstract 643.15.

Thorne et al (2008). *In vivo* diffusion of lactoferrin in brain extracellular space is regulated by interactions with heparan sulfate. ***Proceedings of the National Academy of Sciences*** 105:8416-8421.

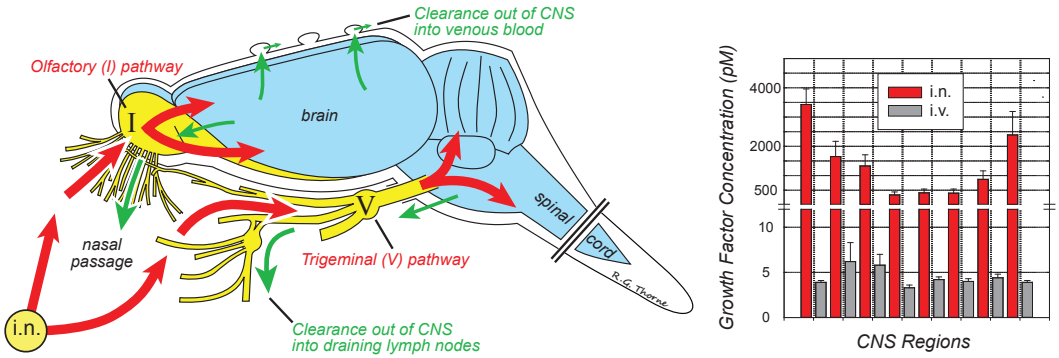
Thorne & Nicholson (2006). *In vivo* diffusion analysis with quantum dots and dextrans predicts the width of brain extracellular space. ***Proceedings of the National Academy of Sciences*** 103:5567-5572.

Thorne, Hrabetova & Nicholson (2005). Diffusion measurements for drug design. ***Nature Materials*** 4:713.

Thorne, Hrabetova & Nicholson (2004). Diffusion of epidermal growth factor in rat brain extracellular space measured by integrative optical imaging. ***Journal of Neurophysiology*** 96:3471-3481.

Thorne & Frey (2001). Delivery of neurotrophic factors to the central nervous system: Pharmacokinetic considerations. ***Clinical Pharmacokinetics*** 40:907-946.

PROJECT 2 - INTRANASAL TARGETING OF DRUGS TO THE CNS. The delivery of potentially therapeutic levels of protein tracers and growth factors to the rat brain from the nasal passages was first described over a decade ago (Thorne et al, 1995; Frey et al, 1997). Many peptides and proteins have since been demonstrated to bypass the blood-brain and blood-cerebrospinal fluid barriers to reach or have effects in the CNS of rodents, monkeys and even humans following intranasal administration (e.g. see Thorne & Frey, 2001; Liu et al, 2001; Ross et al, 2004; Thorne et al., 2004 and 2008). The intranasal route has many advantages for clinical use: non-invasiveness, ease of application/termination, avoidance of hepatic first-pass elimination, and a growing record of experience with approved formulations (e.g. nasal spray of the 3.5 kDa polypeptide hormone calcitonin has been used to treat postmenopausal osteoporosis for many years). However, the mechanisms involved in direct transport to the brain from the nasal passages are not yet clear (Thorne, 2009). Our research suggests rapid, extracellular flow occurs along olfactory and trigeminal nerve components in the nasal epithelium to the olfactory bulb and brainstem, respectively, where dispersion to other CNS areas may be possible via pulsatile flow within the perivascular spaces of cerebral blood vessels (Thorne et al, 2004 and 2008). Our aim is to identify precisely how proteins, viruses, nanoparticles and even cells appear to be able to cross the nasal epithelial barriers and then widely distribute within the CNS. Our work in this area uses fluorescent, radiolabeled and electron dense compounds along with several different imaging and quantification methods, some of which overlap with *Project 1*. The findings will greatly aid in the application of this method; indeed, clinical trials to treat developmental disorders, neurodegenerative diseases and stroke are just beginning.



Intranasal (i.n.) administration provides access to olfactory and trigeminal pathways (shown in red for the rat), allowing growth factors, nanoparticles and cells to potentially reach CNS regions in concentrations orders of magnitude higher than after traditional systemic routes such as intravenous (i.v.) administration (data from Thorne et al., 2004).

Lochhead & Thorne (2012). Intranasal delivery of biologics to the central nervous system. ***Advanced Drug Delivery Reviews*** 64:614-628.

Lochhead, Wolak & Thorne (2011). Towards a mechanistic understanding of brain delivery following intranasal administration. ***Society for Neuroscience Annual Meeting*** (14 Nov 2011; Wash DC).

Thorne (2009). Nose-to-brain drug delivery? (http://www.pdonlineresearch.org/responses/2763/180/nose-brain-drug-delivery).

Thorne, Hanson, Ross, Tung & Frey (2008). Delivery of interferon-beta to the monkey nervous system following intranasal administration. ***Neuroscience*** 152:785-797.

Thorne et al (2004). Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. ***Neuroscience*** 127:481-496.

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Liu, Fawcett, Thorne, DeFor and Frey (2001). Intranasal administration of insulin-like growth factor-1 bypasses the blood-brain barrier and protects against focal cerebral ischemic damage. ***Journal of the Neurological Sciences*** 187: 91-97.

Frey, Liu, Chen, Thorne, Fawcett, Ala & Rahman (1997). Delivery of 125I-NGF to the brain via the olfactory route. ***Drug Delivery*** 4: 87-92.

Thorne, Emory, Ala & Frey (1995). Quantitative analysis of the olfactory pathway for drug delivery to the brain. ***Brain Research*** 692:278-282.