Bypassing the central nervous system barriers: current state of the art

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Lochhead & Thorne (2012)
Thorne et al. (2004)
Wolak & Thorne (2015)
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(ii) being listed as an inventor on patents and patent applications related to CNS drug delivery

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Utilizing biologics as CNS therapeutics – Where are we today?

- **Systemic approaches have yet to be translated fully to the clinic (no approvals)** – the CNS barriers pose a unique challenge

- **Only 3 FDA-approved CNS biologics are actually delivered into the brain**
  - intrathecal ziconotide (2.6 kDa) / chronic pain
  - intrathecal nusinersen (~7 kDa) / SMA
  - intraventricular cerliponase alfa (59 kDa) / rhTripeptidyl peptidase (N-term)

- **Late infantile neuronal ceroid lipo-fuscinosis type 2 (CLN2) – Batten disease**

- **Many uncertainties remain**
  - precise brain / spinal cord distribution
  - relative effectiveness of different routes
  - if limited delivery, how can we enhance?

- **What is needed?** We need to better understand the mechanisms governing drug delivery and distribution in the CNS
**Diffusive transport of therapeutics in the neuropil**

Brain extracellular spaces (ECS) are 40-60 nm wide

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**Predicted diffusion gradients from *in vivo* diffusion measurements (IgG, 150 kDa) — limited penetration / **NOT** scalable**


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non-targeted IgG; based on *in vivo* brain diffusion coefficient (using integrative optical imaging point source method)

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10 mm
Convective transport of therapeutics in the CSF & PVS

Cerebrospinal fluid (CSF) circulation pathways – scalable across species

Perivascular space (PVS) fluid compartments – may also allow for some circulation / potentially scalable

Adapted from: Thorne. IN: Drug Delivery to the Brain. Springer (2014)

**Perivascular spaces**

- Fluid and connective tissue compartments surrounding subarachnoid & cerebral vessels.

- Large enough (5-10 μm) to allow for flow (convection); arterial pulsations may serve as a driving force.

- Serve a possible lymphatic function.

**Sources:**
Normal adult brain ECS width in vivo ~ 40 – 60 nm

[ Heparan sulfate binding sites ] ~ 3.5 \( \mu \)M


1 – Local transport in brain extracellular space (ECS) after intraparenchymal injection

2 – Whole brain distribution following intrathecal infusion into the cerebrospinal fluid (CSF)

3 – Whole brain distribution after intranasal administration

My laboratory’s focus CNS delivery and distribution of biologics – Study of mechanisms & new strategies for three central routes

CNS distribution resulting from intrathecal infusions

How widespread can it be? What determines the distribution?

Transport at the brain – CSF interface: A delicate mix of diffusion within brain extracellular spaces & convection within perivascular spaces
Intrathecal infusions — Imaging I.T. IgG distribution in rats reveals the critical role of perivascular flow

MRI of I.T. Gd-IgG

Ex vivo fluorescence imaging of I.T. AF488-lgG

Left panel: Lochhead et al. JCBFM (2015); Right panel: T1 MRI – baseline subtracted (visualization using ImageJ); 50 min infusion + post-infusion imaging.
**Intrathecal infusions** – 1. antibody size-dependence
2. the balance between diffusion & perivascular flow
3. distribution enhancement by co-infusion of mannitol

**AF488-sdAb – ~15 kDa**

**AF488-IgG – 150 kDa**

- goat IgG + 0.27 M mannitol
- goat IgG + 0.75 M mannitol

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Pizzo, … & Thorne. *Journal of Physiology, in press* (2017); AF488-labeled A20.1 VHH, llama sdAb provided by Dana Stanimirovic (NRC, Canada)
**Intranasal targeting to the brain**
What determines the distribution?

1. **Transport across nasal epithelia to reach brain entry pathways or blood vessels (BV) for systemic absorption**

2. **Transport along olfactory & trigeminal pathways from the nasal mucosa (NM)**
   - Olfactory nerve-associated pathway
   - Trigeminal nerve-associated pathway

3. **Widespread distribution in the brain via flow within perivascular spaces associated with major cerebral arteries**

**Intranasal**
- PBS (control)
- TR-dextran (3 kDa)

**IGF-I** (7649 Da; 70 aa)

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From: Lochhead et al. JCBFM (2015); Reviewed in: Lochhead & Thorne. ADDR (2012)

Conclusions

- **Diffusive transport** of large macromolecules (e.g. enzymes) into the brain from the CSF will be **quite limited** (several mm)

- Access to & distribution within the perivascular spaces will likely be **critical** for widespread distribution

- There is an urgent need to understand all key variables
  - body position
  - intracranial pressure
  - co-applied excipients (e.g. osmotic methods)
  - disease / storage effects
  - individual variation

Top right: Ziegler et al. *Exp Neurol* (2011); Other images – Pizzo, Kumar & Thorne. Submitted / Unpub. + collab. w/ Lydia Sorokin (Univ. of Muenster, Germany)