The Glymphatic System, Sleep & other states of unconsciousness

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Significance

• The brain is one of the most metabolically active organs in the body

• Removal of excess fluids and waste products is critical for normal brain function

• The brain parenchyma has no authentic lymphatic vessels for detoxification
The brain & metabolic waste: Example - Ammonia (NH₃)

The glutamate-glutamine cycle leads to production of ammonia (NH₃)....at the same time ammonia is needed in astrocytes for the synthesis of glutamine from glutamate.

Ammonia is rapidly eliminated by the brain

From: Schousboe et al. Frontiers in Endocrinology, 2013
The brain & metabolic waste: Proteins

- Cytosolic proteins are constantly renewed
- Proteins incorporated in the cytoskeleton and membranes are constantly renewed
- Lack of specialized BBB transporters for most peptides and protein
- Recycling/degradation of protein: ubiquitination and autophagy

Schematic overview of autophagy
Rubinzstein, NATURE, Vol 443; 206
Significance

• Age-related decline in the efficiency of protein degradation has been implicated in pathological protein aggregation

• Neurodegenerative diseases, are characterized by accumulation of aggregation-prone mutated, misfolded or hyperphosphorylated proteins

• These proteins are present intracellular and extracellular

Neurofibrillary tangle and amyloid plaque

http://neuropathology-web.org/
Alternative waste removal pathway from CNS: CSF-ISF exchange

New discovery (mouse), 2015

Brain parenchyma lacks a lymphatic system?

CSF has been proposed as a sink for waste removal from brain

Louveau et al., Nature 2015

CSF is produced by the choroid plexus in the ventricles from where it flows through foramen magendi and eventually leaves via the arachnoid villi, the olfactory bulb, or cranial nerves....
Waste removal via CSF characterized by Heinrich Quincke in 1872

Dr. Quincke

Quincke injected **cinnabar granules** into CSF of anim. (dogs, cats, rabbits)

Cinnabar is a large molecule and while it is transported in CSF it does not go into parenchyma....

From: “Modern CSF Research and Heinrich Quincke’s Seminal Paper on the Distribution of Cinnabar in Freely Moving Animals. Benveniste et al., JCN 2015
State-of-the-art knowledge of the glympathic pathway
State-of-the-art knowledge of the ISF-CSF exchange process

A594 MW 759 Da – Red
TR-d3 MW 3000 Da – Blue
FITC-d2000 MW 2000kDa - Green

Illif et al., Science Translational Medicine, 2012
In vivo imaging of CSF tracers: Discovering the glymphatic pathway

Vasculature
- CSF tracer
- 180 µm below the surface
- 5-10 min

Iliff – Nedergaard (et al.), Science Translational Medicine, 2012
How do we know that this system removes waste products?

Iliff et al., Science Translational Medicine, 2012
State-of-the-art-knowledge:

Visualizing glymphatic transport in real time using MRI
Tracking glymphatic transport using contrast-enhanced MRI

3D FLASH sequence (T1-weighted); each scan acquired over 4 min

%Signal change from baseline

Whole Brain, N=9

Iliff, Nedergaard, Benveniste; J Clin Invest. 2013 Mar 1;123(3):1299-309
Quantification of Gd-DTPA brain-wide transport

![Graph showing %Signal Change from baseline over time for Whole Brain and Cisterna Magna](image)

**2-Compartment Model**

- **Cistera Magna**
- **C1**
- **C2**

- $K_1$:
- $k_2$
- $k_3$
- $k_4$

- "Retention" = $k_3/k_4$
- "loss" = $k_2/(1+k_3/k_4)$

Lee et al., J Neuroscience. 2015
Brain loss (clearance) and ‘retention’ of Gd-DTPA after CSF administration in rodent whole brain

Lee et al., J Neuroscience 2015
Factors that influence glymphatic pathway function:

- AQP4
- Adrenergic tone
- Pulsatility
- Sleep / hypnotics
Wakefulness suppresses influx of CSF tracers

Importance of Noradrenergic tone


..We also see differences in glymphatic transport with use of different anesthetics...

Alpha-2 agonist - dexmedetomidine
Knowledge gap:

If unconsciousness enhance brain waste removal – what about body position during the unconscious state?
How does body position during sleep/anesthesia influence glymphatic transport?

“Consistently, poor sleepers spent more time on their backs with their heads straight” De Koninck et al., Sleep 1983;6 (1):52-9

The most favored position is right lateral decubitus (Sleep 1983;6 (1):52-9)

http://bestadjustablemattress.com/how-to-find-the-best-sleeping-positions/
Results from DeKonick’s paper

<table>
<thead>
<tr>
<th></th>
<th>Good Sleepers</th>
<th>Poor Sleepers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body movement/night</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Film records</td>
<td>42.3</td>
<td>50.5</td>
</tr>
<tr>
<td>Polygraph records</td>
<td>56.7</td>
<td>77.0</td>
</tr>
<tr>
<td>Position changes/night</td>
<td>22.3</td>
<td>35.6</td>
</tr>
<tr>
<td>By trunk position (first 3 cycles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Back</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Right side</td>
<td>24</td>
<td>17</td>
</tr>
<tr>
<td>Left side</td>
<td>15</td>
<td>11</td>
</tr>
</tbody>
</table>

Average body movements and trunk position for good and poor sleepers for both nights (modified Table 4 from (Sleep 1983;6 (1):52-9))
Sleeping gorillas

“Slow wave sleep (SWS). During this stage, subjects assumed a reclining posture, sometimes on their abdomen....sometimes curled in a fetal position with eyes closed.” (Hobson; Behavioral Neuroscience; 2000, Vol 114; No. 6, 1239-1244).

Effect of body position on glymphatic transport

Neck, ears and nose is not affected by the head holder

MRI compatible head holder fixing the head of the anesthetized rat in prone position during imaging. There are no ear-bars; the fixation points are at the level of the squamosal bone. Head holder designed by Dr. Hedok Lee.
Physiological parameters:

Anesthetized rat in the SUPINE position being monitored: Respiratory rate, heart rate, Oxygen saturation and body temperature.

All the rats were anesthetized with a mixture of Ketamine:Xylazine i.p.; glycopyrulate 0.01mg/kg i.p was also administered as an anti-sialagogue. All rats were spontaneously breathing.
2-compartment model

Clearance (loss) is greatest in RLD and lowest in prone position...

\[ k_3/k_4 = \text{Retention} \]
\[ k_2/(1+k_3/k_4) = \text{“loss”} \]
Does body position influence whole brain glymphatic transport of Gd-DTPA

Data are presented as median (1st quartile, 3rd quartile) for each group. *One rat in the PRONE group was excluded from analysis due to 2-compartmental fitting failure.

<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>SUPINE (N=9)</th>
<th>PRONE (N=6*)</th>
<th>RLD (N=8)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention = (k3/k4)</td>
<td>10.70 (9.88, 12.50)</td>
<td>14.98 (12.23, 16.53)</td>
<td>6.86 (6.28, 9.47)</td>
<td>0.008</td>
</tr>
<tr>
<td>Loss = k2/(1+k3/k4)</td>
<td>0.23 (0.13, 0.26)</td>
<td>0.14 (0.09, 0.21)</td>
<td>0.31 (0.23, 0.40)</td>
<td>0.021</td>
</tr>
</tbody>
</table>
MRI data validated by optical imaging....and also showing that $A\beta$ is cleared least efficiently in prone position
Research opportunities

• Understand how the cardiac sympatho-vagal balance influence central nervous system arousal and glymphatic pathway function in the context of sleep and aging.

• Understand how perivascular neurons, gliovascular interactions and intramural vascular signaling change and interferes with glymphatic pathway functioning in normal aging.

• Mapping of the glymphatic, perivascular ‘connectome’; need to understand CSF-ISF streaming pattern in health and disease.