

Network dysfunction underlying cognitive impairment in repetitive mild traumatic brain injury (TBI)

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Background

Traumatic brain injury (TBI)

- Traumatic brain injury (TBI):** alteration in brain function or brain pathology caused by an external force
- 50-60 million new cases globally every year
 - Over 90% are mild but result in **impaired attention, learning and memory** in the **subacute/chronic phase** (> 6 months post-injury)¹
 - Currently no effective treatment for cognitive deficits
 - **Mild, repetitive TBI (rmTBI)** is common amongst **athletes, abuse victims and veterans**
 - Conventional structural neuroimaging scans appear normal as they are not sensitive to microscale tissue damage, hence the need for **animal models** where we have access to **high resolution and sensitive** assays

tACS as a potential treatment

- **Transcranial alternating current stimulation (tACS)** is a non-invasive neuromodulation technique
- Only superficial cortical regions are directly stimulated but **effects propagate through brain networks**
- Able to **improve performance** across multiple cognitive domains in **healthy subjects** but **highly variable**²
- Current tACS paradigms for TBI are **borrowed from other diseases** rather than being informed by the connectivity state of individual patients

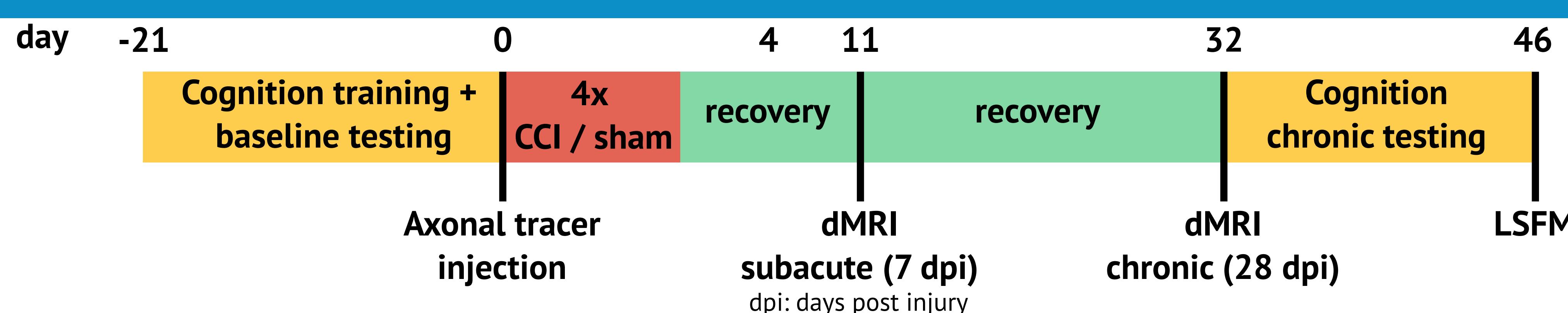
Aim

Characterize subacute and chronic functional and structural network dysfunction underlying TBI-induced cognitive impairment to optimize tACS treatment for TBI.

Hypothesis

Dysfunctional connectivity within and between sensory cortices, hippocampus and prefrontal cortex will correlate with cognitive performance.

Methods



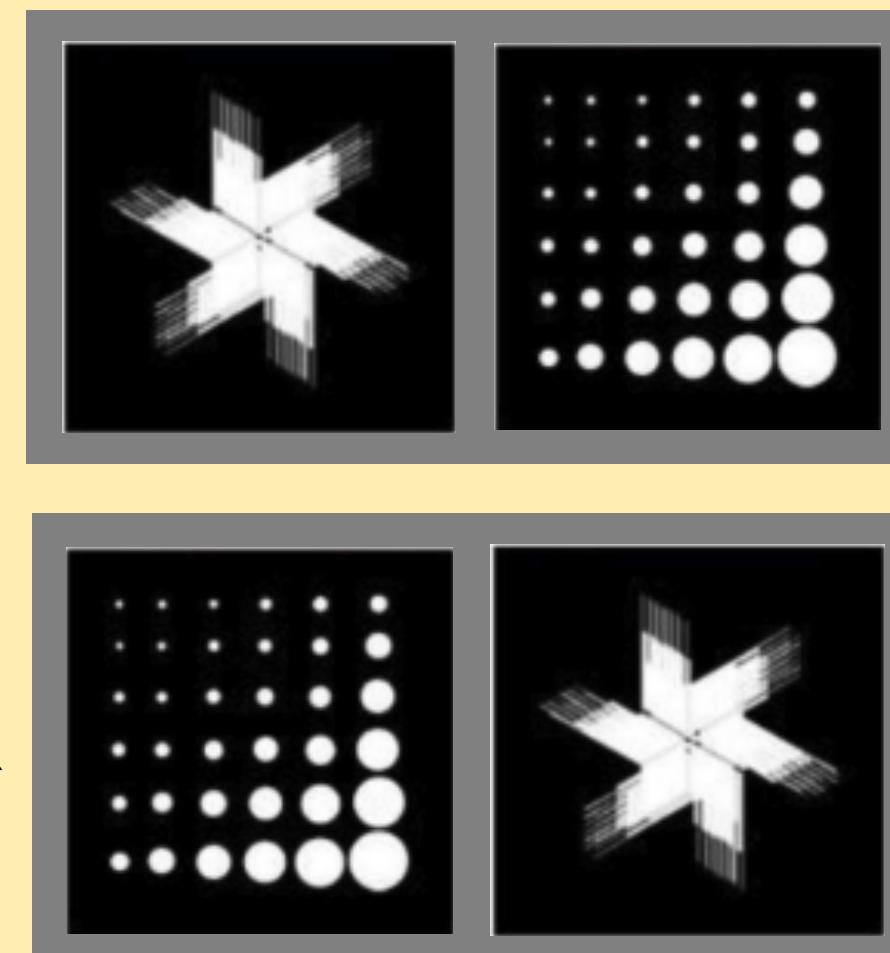
Touchscreen operant chambers

- ✓ Similar to human cognition tests
- ✓ Less stressful than conventional mazes
- ✓ Quantitative evaluation of task strategy



Paired visual discrimination (PWD) and reversal (PWD-R)

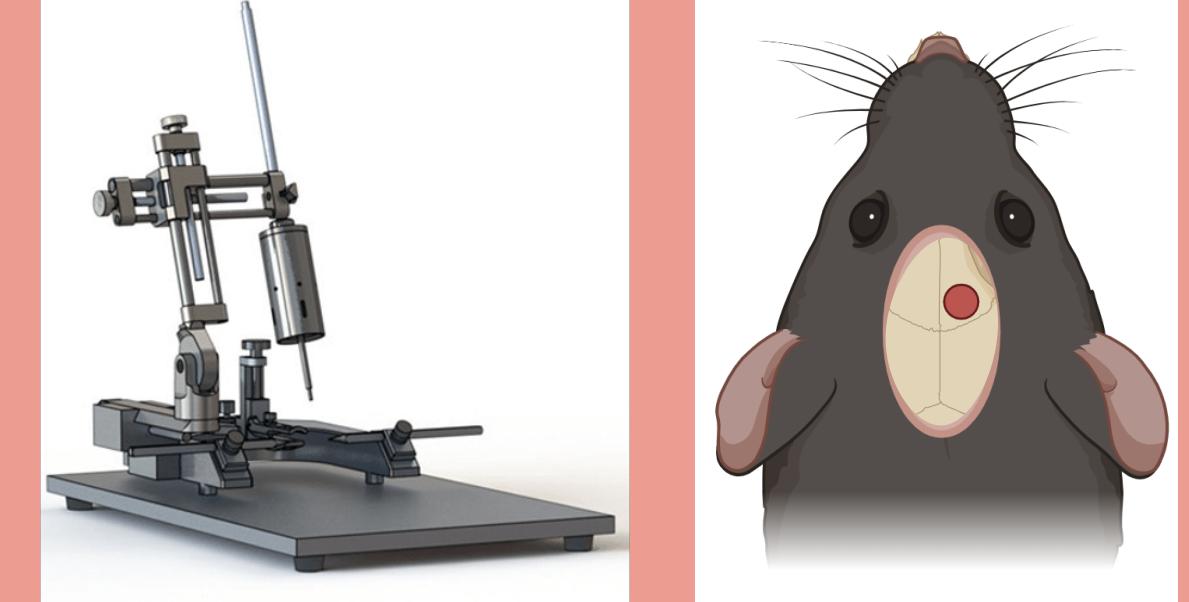
correct wrong



Mice learn to nose-poke the correct stimulus (fan) in the PWD test to 80% accuracy in a 30 trial session. After TBI/sham surgery, mice are tested again on the PWD test before reversing the stimuli such that the marbles image is now the correct stimulus. Mice must achieve 80% accuracy in a 30 trial session prior to the probe test on the PWD-R.

PWD tests for **visual reference memory** while PWD-R tests for **cognitive flexibility**³.

Controlled cortical impact (CCI)



- ✓ Reproducible and consistent hit
 - ✓ Biomechanically similar to human mild TBI⁴
- Parameters:**
4 hits 24h apart
4.0 m/s velocity
1mm impact depth
3mm impact diameter over motor or somatosensory cortex

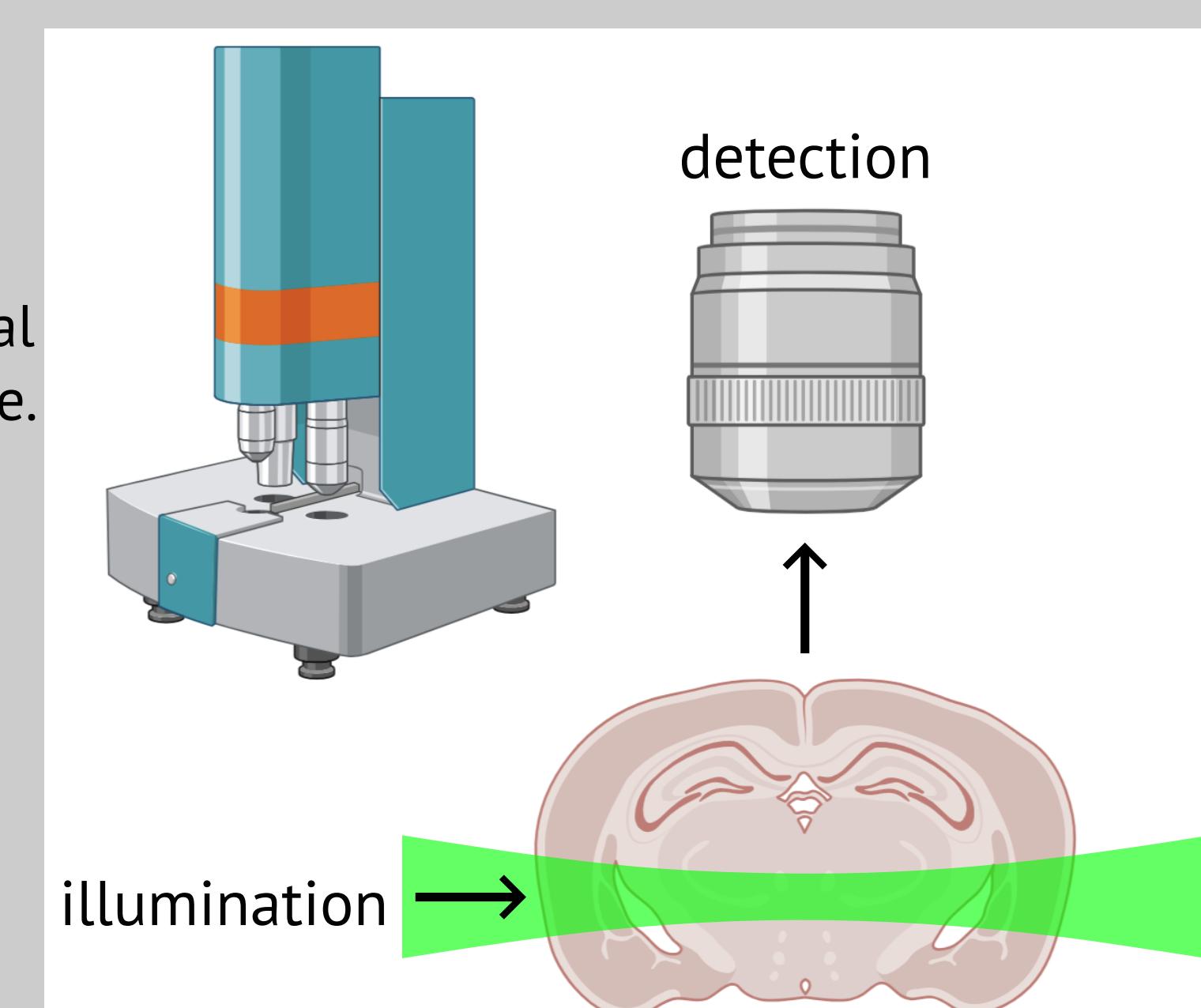
Axonal tracing

AAV8-DJ/8.CaMKIIa.EYFP.NRN injected at injury site after the first impact to label axonal projections, allowing characterization of structural connectivity changes of the injury site.

Lightsheet fluorescence microscopy (LSFM)

After completion of cognition testing at the chronic stage, brain tissue was collected and optically cleared via SHIELD for imaging with LSFM.

- ✓ Minimal photobleaching
- ✓ Optical sectioning
- ✓ Rapid whole-brain imaging

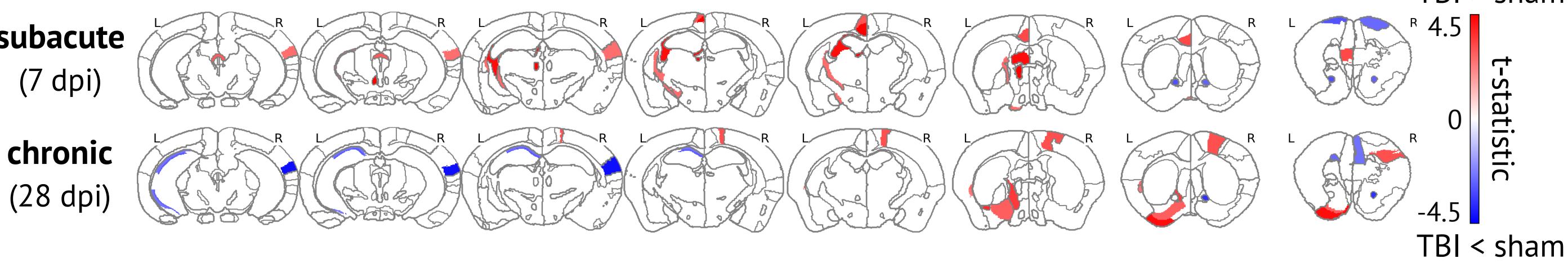


diffusion weighted MRI (dMRI)

dMRI was acquired in 33 directions at b values 1215 and 1928. Preprocessing was performed in FSL and MRtrix prior to diffusion tensor fitting via the DTIFIT toolbox. Individual scans were registered and parcellated into 336 regions based on the DSURQE atlas.

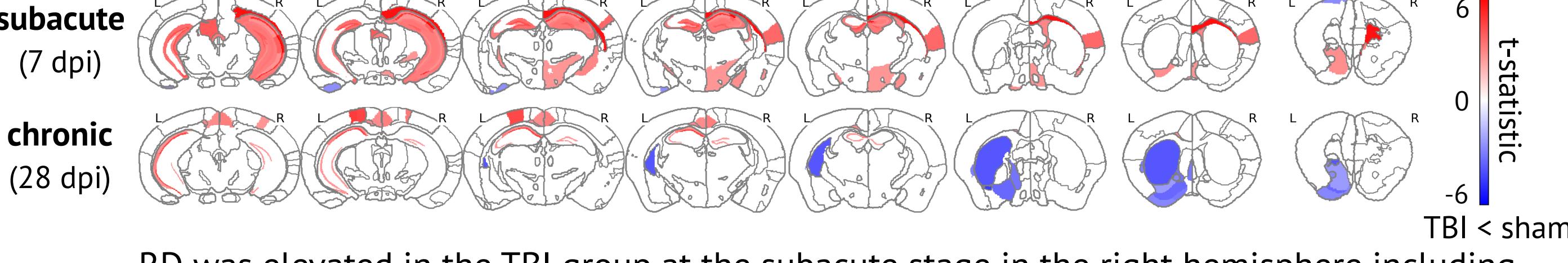
dMRI Results

Fractional anisotropy (FA) - associated with white matter integrity



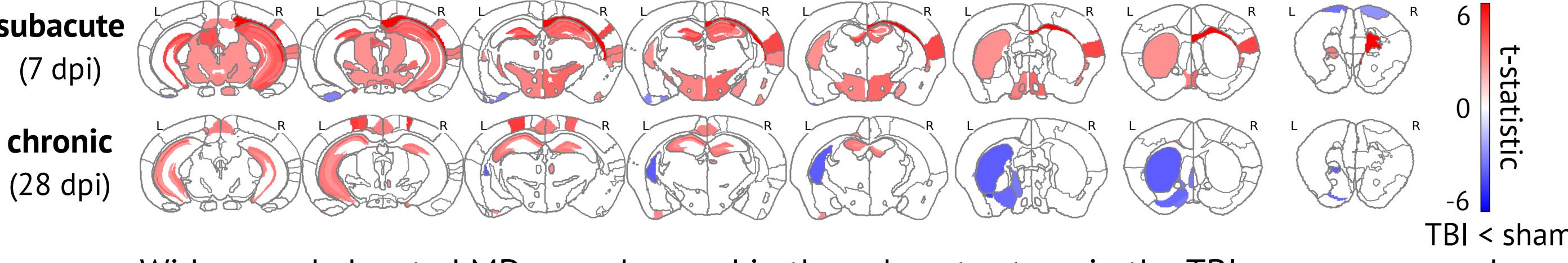
Increased FA in some grey matter areas were observed in TBI compared to shams.

Radial diffusivity (RD) - associated with myelination



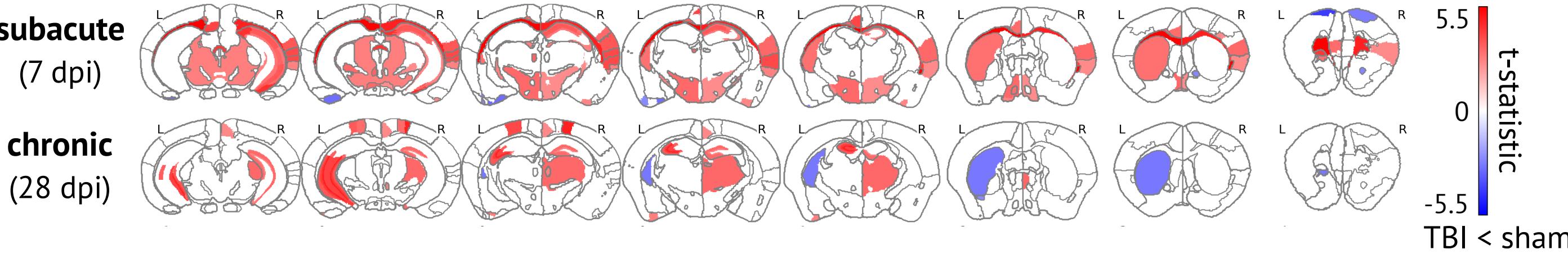
RD was elevated in the TBI group at the subacute stage in the right hemisphere including the corpus callosum and hippocampal regions. At the chronic stage, the elevated RD in the right hemisphere normalized while more hippocampal and cortical regions in left hemisphere exhibited elevated RD.

Mean diffusivity (MD) - associated with edema, cell infiltration



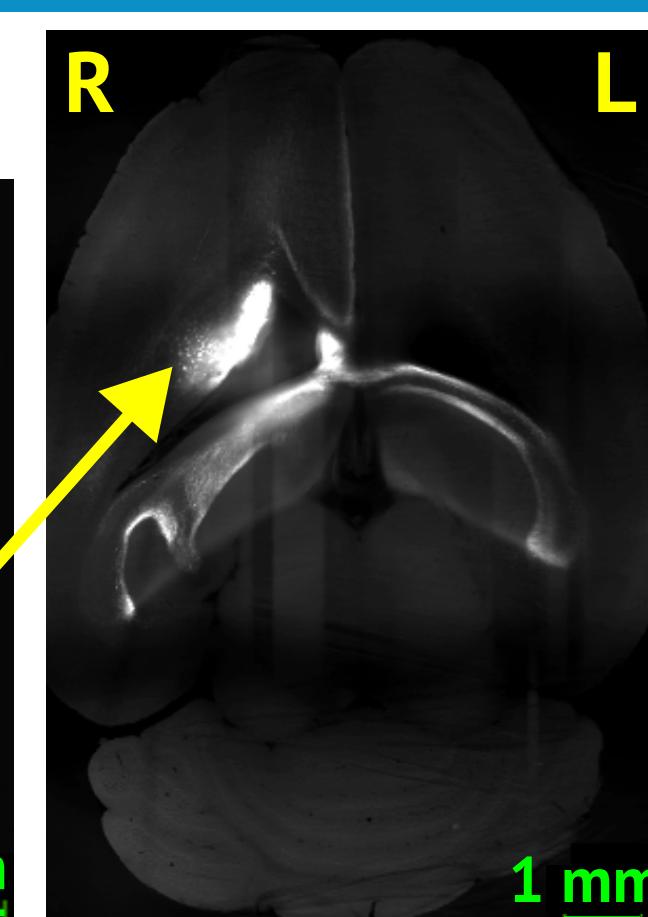
Widespread elevated MD was observed in the subacute stage in the TBI group compared to the sham group which normalized in most regions at the chronic stage but remained elevated in several hippocampal subregions.

Axial diffusivity (AD) - associated with white matter integrity



AD was elevated in the TBI group at the subacute stage in right grey matter regions and bilaterally in the corpus callosum. At the chronic stage, AD in the corpus callosum normalized but was elevated in cortical regions in the TBI group.

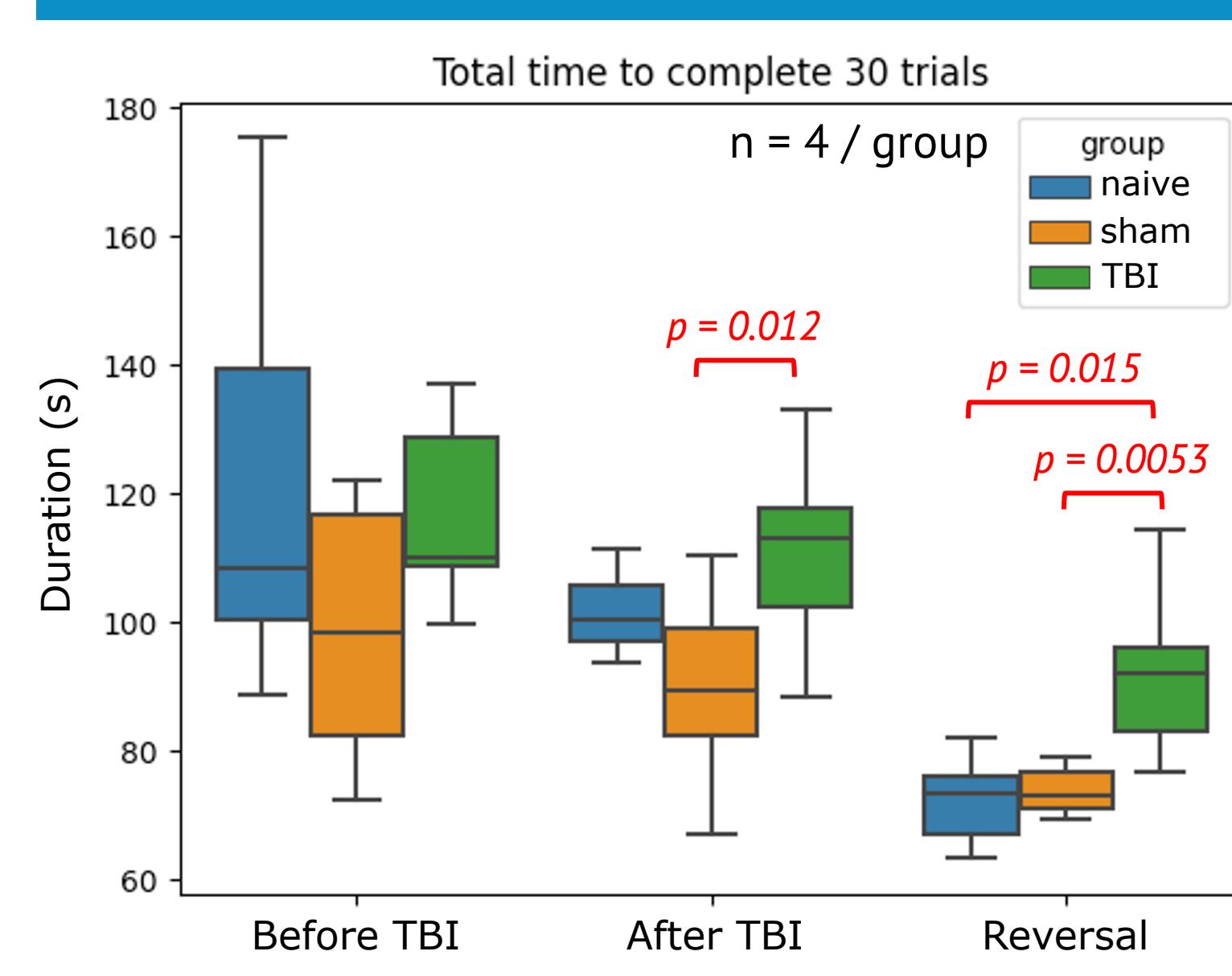
Axon Tracing Results



- Axons projected from the injury site to:
- ipsilateral cortex
- ipsilateral hippocampus (more)
- contralateral hippocampus (less)
- ipsilateral striatum

LSFM images will be registered to an atlas to obtain parcellations and perform axon segmentation to quantify changes in axonal density of various regions due to TBI.

Cognition Results



After TBI, mice took **significantly longer** than shams to **complete the PWD and PWD-R session**. All mice showed improvement in completion time but mice in the **TBI group displayed a smaller degree of improvement**.

Conclusion & Future Work

Conclusion

1. TBI likely **impaired attention, learning and visual reference memory**.
2. The CCI causes **demyelination** (indicated by RD) **subacutely in the ipsilesional hemisphere** which **normalizes chronically**. Demyelination in the **contralateral hemisphere** is low subacutely but **persists chronically**.
3. CCI was expected to reduce AD due to axonal injury but **AD was instead elevated after TBI**, particularly in the **corpus callosum**. This could be due to immune cell infiltration, edema or axonal swelling^{5,6}, which is supported by the increase in MD.

Future work

1. Investigate the **cause of elevated AD** by comparing DTI findings with axon tracing data and immune cell histology from LSFM.
2. Identify **associations between cognition readouts and dMRI metrics** of various brain regions
3. Characterize **functional connectivity** changes.
4. Test other cognitive paradigms to **more specifically identify the impaired cognitive domain**.

References

1. McInnes et al. (2017) PLoS One; 2. Grover et al. (2013) Sci. Transl. Med.
3. Horner et al. (2013) Nat. Protoc.; 4. Chen et al. (2014) Front. Neurol.;
5. Budde et al. (2009) J. Neurosci.; 6. Mac Donald et al. (2007) J. Neurosci.