Psilocybin as a treatment for microstructural and functional alterations following repetitive mild head injury: A preclinical MRI study

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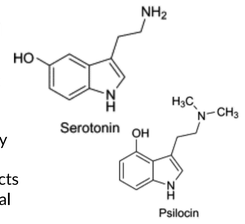
Introduction

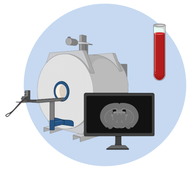
Repetitive Mild Traumatic Brain Injuries (rmTBI), commonly known as concussions, pose a significant risk factor for neurodegenerative disease onset later in life. Psilocybin, a psychedelic 5-HT2A agonist, has recently garnered interest as a robust promoter of neuroplasticity with synaptogenic and anti-inflammatory effects. Our study is the first to investigate the neuroprotective effects of psilocybin as a treatment for rmTBI using a multimodal preclinical approach.



Experimental Design

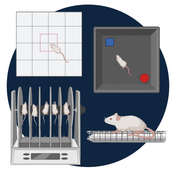
rmTBI & Psilocybin Treatment

- **Day 0:** Adult female Wistar rats (*N*=24) assigned to groups: Sham rmTBI + Vehicle, rmTBI + Vehicle, rmTBI + Psilocybin
- **Day 1-3:** Extended-release buprenorphine (0.1 mg/kg) analgesic treatment → mTBI or Sham → 20 min. post-injury Psilocybin (3.0 mg/kg) or Vehicle → 20 min. head twitch response recordings to indicate psychoactive dosing
- **Day 2:** mTBI or Sham → Psilocybin or Vehicle → Twitch
- **Day 3:** mTBI or Sham → Psilocybin or Vehicle → Twitch …



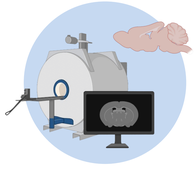
Post-Injury Neuroimaging & Peripheral Biomarkers

- **Day 3 (continued):** 20 min. post-treatment blood plasma collection via lateral tail vein to quantify psilocybin, psilocin, and lipid biomarkers of mTBI, followed by neuroimaging
- 7.0 T Magnetic Resonance Imaging: T2-Weighted & Diffusion Weighted Imaging



Behavioral Assays

- **Day 4:** Open Field test of exploratory locomotor behavior
- **Day 5:** Novel Object Recognition test of short-term memory
- **Day 10:** Rotarod and Tapered Balance Beam tests of motor coordination and endurance

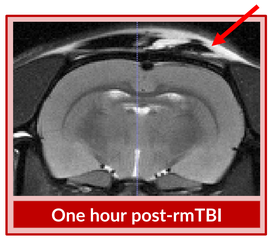


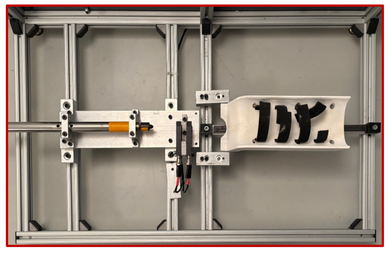
Follow-Up Neuroimaging & Tissue Analysis

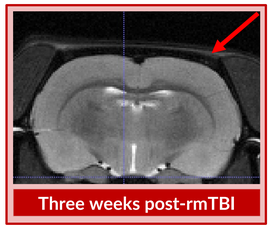
- **Day 15-19:** Acclimation for awake functional MRI (fMRI)
- **Day 22:** T2-Weighted, Functional Connectivity, fMRI with Hypercapnic Challenge, and Diffusion Weighted Imaging
- **Day 23:** Tissue collection for proteomics and immunohistochemical analysis of astrocytes (GFAP), microglia (IBA1), pre-tangle tau (MC1), phosphorylated tau (PHF-1), and phosphorylated TDP43

Momentum Exchange Model

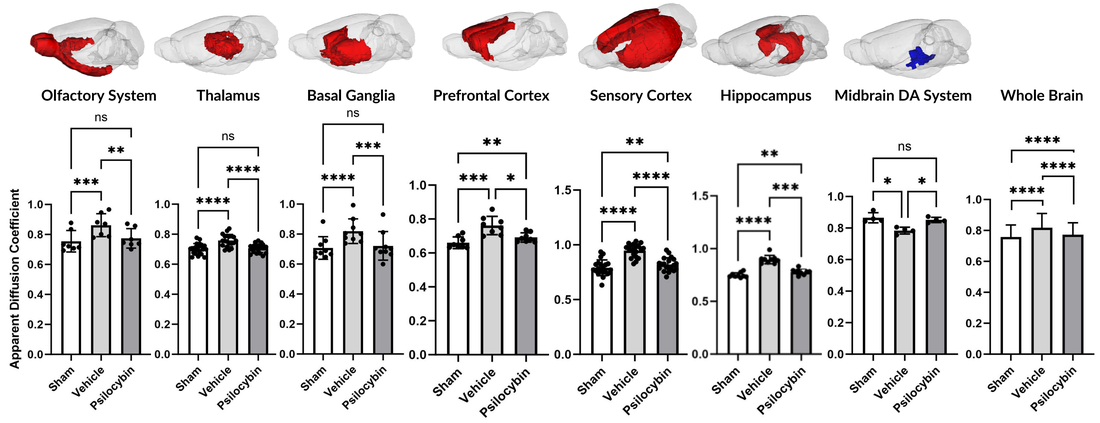
The momentum exchange method ranks highly among preclinical mTBI models in translational value for its ability to generate tightly regulated, ecologically valid closed-head injury in awake, active-phase rats. Upon impact (7.4 m/s), the cradle accelerates backward along its track. Radiography indicates no skull fracture or gross anatomical damage. Transient superficial edema of the tissue overlying the skull at the site of impact is shown on the third day of rmTBI.







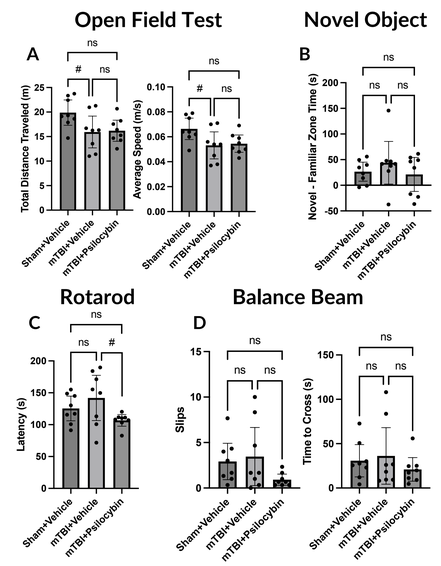
Acute Administration of Psilocybin Prevents Early Peak in Neuroinflammation Induced by rmTBI



Diffusion Weighted Imaging (DWI) conducted on Day 3 (within one hour of the final mTBI and psilocybin treatment) indicates significant changes in gray matter microarchitecture globally and regionally. Tissue diffusivity was broadly increased throughout the forebrain and decreased throughout the midbrain, indicating vasogenic and cytotoxic edema in response to rmTBI; however, these trends were prevented or mitigated by psilocybin treatment. Three weeks later, no group differences were identified, suggesting microstructural injury recovered and was not impeded by psilocybin.

Non-parametric Kruskal-Wallis test: n.s. no significance; * p<0.05; ** p<0.005; *** p<0.0005; **** p<0.00005

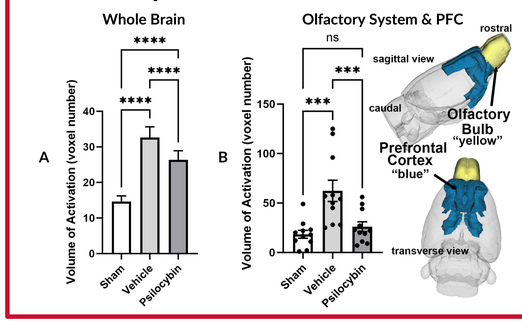
Cognitive and Motor Assessment



Consistent with prior reports of the momentum exchange model of mild head injury, few behavioral effects were observed in the first week post-rmTBI. Open Field testing on Day 4 indicated a trend toward significantly reduced locomotor exploration in both injury conditions **(A)**. Rotarod testing on Day 10 indicated a trend toward significantly reduced latency in the psilocybin condition **(C)**, however tapered balance beam walk results on the same day indicate this trend is likely not indicative of motor skill impairment **(D)**.

One-Way ANOVA
n.s. no significance
p ≤ 0.057

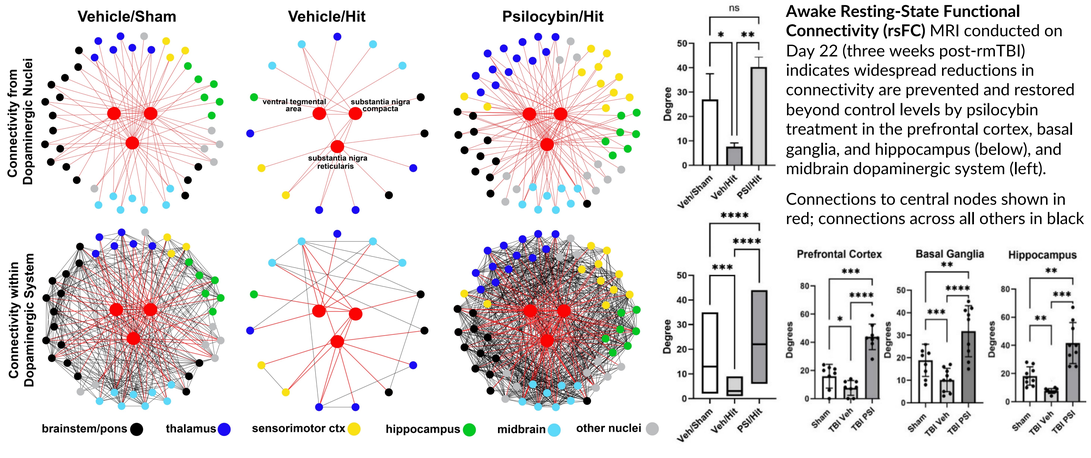
Psilocybin Treatment Limits Lasting Alterations to Neurovascular Coupling



Functional Magnetic Resonance Imaging (fMRI) was conducted in awake rats on Day 22 (three weeks post-rmTBI) with a hypercapnic challenge. As a measure of neurovascular coupling, blood-oxygen-level-dependent (BOLD) signal changes from baseline were recorded during exposure to 5% CO_2 inhalation. Significant increases in the volume of positive BOLD activation throughout the brain indicates a compensatory increase in the vascular reactivity of untreated rmTBI rats relative to untreated sham rats. Critically, this lasting functional alteration is limited globally in psilocybin-treated rmTBI rats **(A)** and prevented entirely in the olfactory system and prefrontal cortex of psilocybin-treated rmTBI rats **(B)**.

Non-parametric Kruskal-Wallis test: n.s. no significance; * p<0.05; ** p<0.005; *** p<0.0005; **** p<0.00005

Psilocybin Treatment Prevents Extreme Loss of Functional Connectivity



Awake Resting-State Functional Connectivity (rsFC) MRI conducted on Day 22 (three weeks post-rmTBI) indicates widespread reductions in connectivity are prevented and restored beyond control levels by psilocybin treatment in the prefrontal cortex, basal ganglia, and hippocampus (below), and midbrain dopaminergic system (left).

Connections to central nodes shown in red; connections across all others in black

Discussion

Our study suggests that psilocybin, a psychedelic 5-HT2A agonist, may offer short-term and long-term benefits for brain health following repetitive mild traumatic brain injury, aligning with its known role as an effector of neuroplasticity. Further analysis is needed to elucidate the mechanisms underlying the observed changes in tissue diffusivity, neurovascular coupling, and functional connectivity, and future research must explore the prevention of long-term neurodegeneration. Taken together, our findings contribute to the growing body of evidence supporting the therapeutic use of psilocybin and provide the first preclinical evidence demonstrating its potential as a treatment for repetitive mild traumatic brain injury.

Future Directions

Preliminary analyses

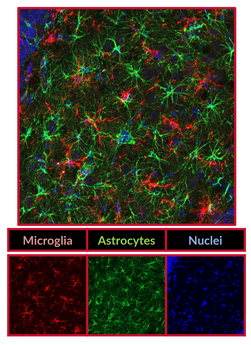
- Day 23 Proteomics: Psilocybin treatment reduces or prevents rmTBI-driven increases of key early indicators of subsequent neurodegeneration throughout the forebrain and midbrain, including phosphorylated tau (PHF-1) and phosphorylated TDP43

Further analyses

- Day 3 Blood Plasma Lipidomics
- Day 23 Immunohistochemistry: Morphology of neuroinflammatory cells (shown at right) and neurodegenerative disease precursors (PHF-1, MC-1, and pTDP43)

Future Studies

- Include males for examination of sex differences
- Test older and adolescent age populations
- Test effects of delayed treatment or microdoses
- Conduct behavioral assays of executive function



Acknowledgments

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