Dose-dependent changes in global brain activity and functional connectivity following exposure to psilocybin: a BOLD MRI study in awake rats

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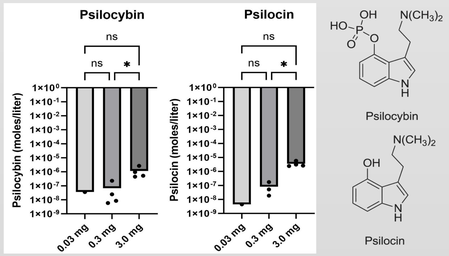
Introduction

Amidst the War on Drugs in 1971, the United Nations classified psilocybin (PSI) and other psychedelic drugs as Schedule 1 substances. However, in the past decade, there has been a resurgence of scientific interest in PSI. Small clinical trials report promising results in treating MDD, end-of-life distress, PTSD, and alcoholism. Animal studies report that low doses of PSI act through 5HT2a receptors to decrease anxiety and promote prosocial behavior, while higher doses also alter dopaminergic signaling, causing cognitive dysfunction. How does PSI alter brain neural circuitry to affect behavior? To address this question, we used BOLD imaging to follow changes in brain activity in male and female rats exposed to PSI

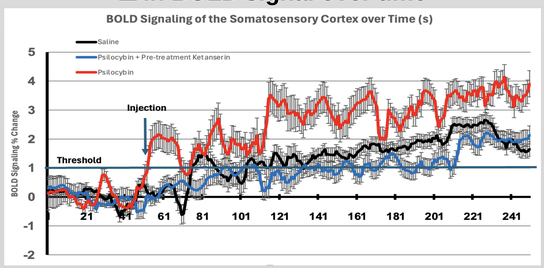
Experimental Design

Female and male rats were given IP injections of vehicle or psilocybin in doses of 0.03, 0.3, and 3.0 mg/kg while fully awake during the imaging session. Changes in BOLD signal were recorded over a 25 min window. Approximately 35 min post injection data for resting state functional connectivity were collected. All data were registered to rat 3D MRI atlas with 173 brain regions providing site-specific changes in global brain activity and changes in functional connectivity. Experiments were conducted under dim red illumination between 10:00 hrs and 18:00 hrs to avoid the transitions between the L-D dark cycles. Blood was assayed for PSI and psilocin 30 min post injection. An additional pilot study tested the effects of the 5HT2A receptor antagonist ketanserin on brain activity in response to the highest dose of psilocybin.

Plasma levels of PSI and psilocin



Δ in BOLD signal over time



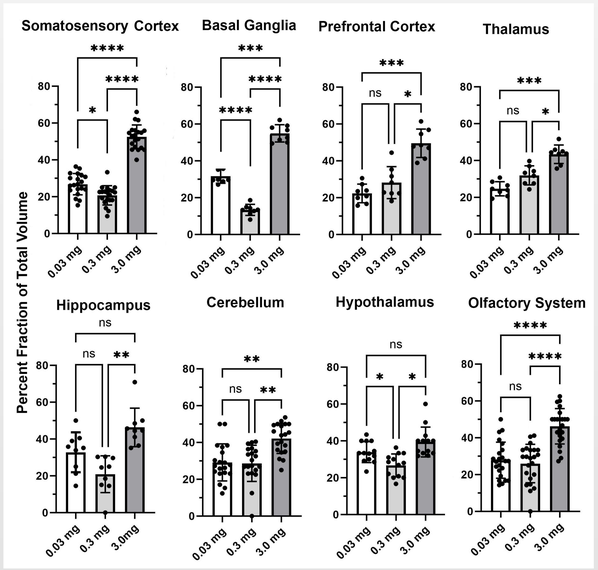
Summary

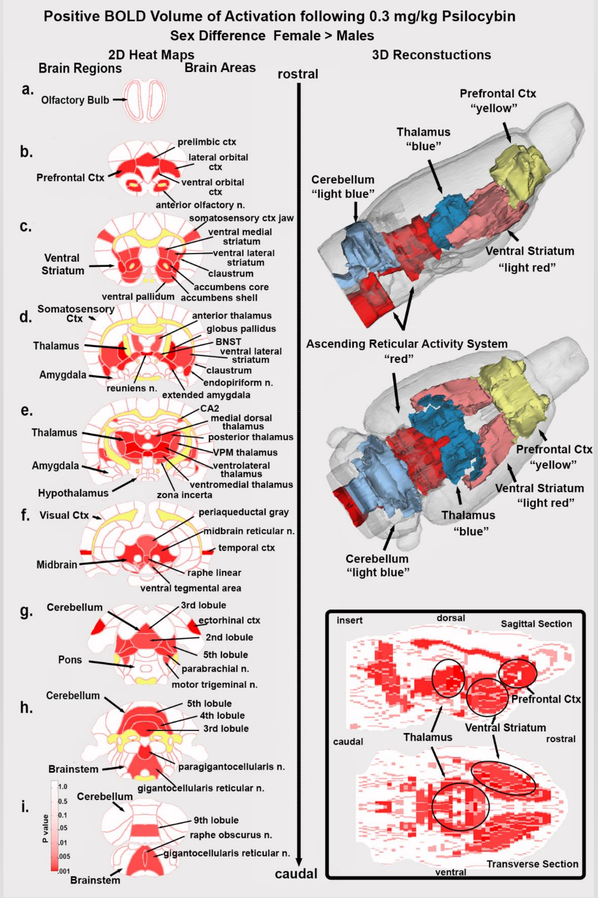
To the best of our knowledge this is the first phMRI study in animals or humans to show a dose-dependent change in BOLD signal in response to PSI. To make these data more relevant to the human condition, imaging was performed while male and female rats were fully awake without the confound of anesthesia and during the dark phase of L-D cycle when rats are normally active. There was a dose-dependent increase in BOLD signal in much of the forebrain, basal ganglia and somatosensory cortex and a global dose-dependent increase in functional connectivity.

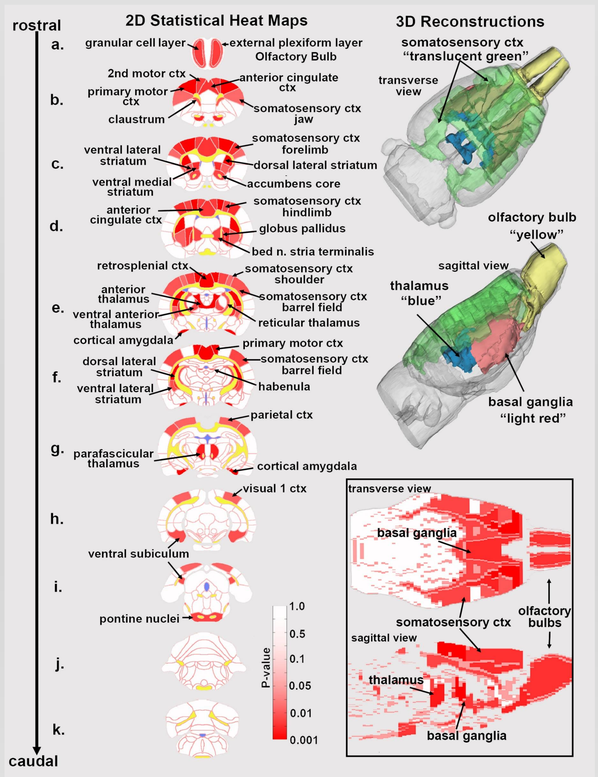
Results

BOLD Pharmacological Functional MRI

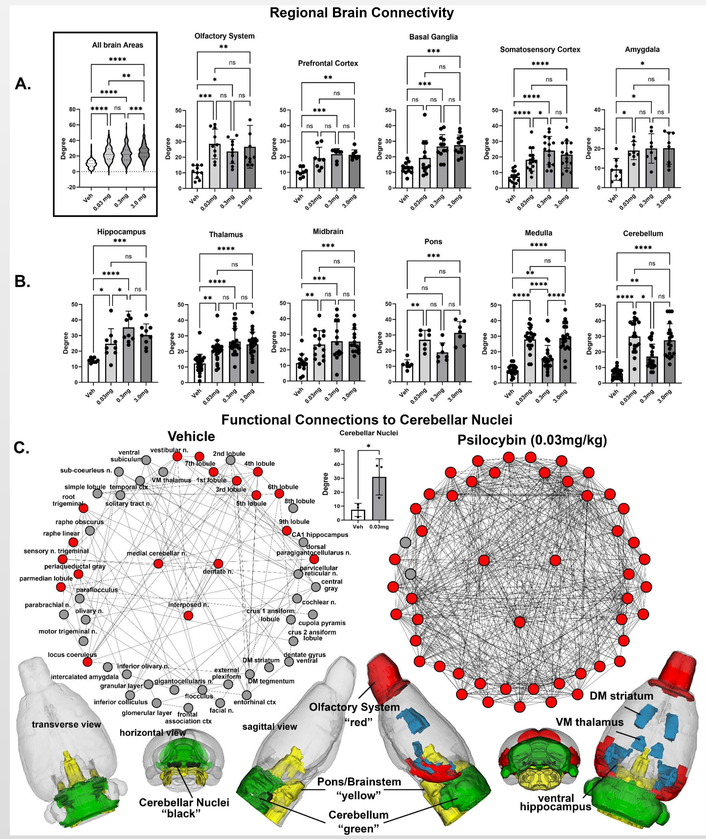
Functional magnetic resonance imaging (fMRI) illustrated that psilocybin (PSI) administration led to a dose-dependent increase in positive BOLD signaling across brain regions, including the prefrontal cortex, somatosensory cortex, basal ganglia, and thalamus. The highest dose (3.0 mg/kg) produced the most significant activation, particularly in the somatosensory cortex and basal ganglia, peaking at 4%. In some regions, such as the primary somatosensory cortex and basal ganglia, a U-shaped dose response was observed, with the 0.3 mg/kg dose showing the lowest activation. A total of 33 brain areas demonstrated significant activation across different PSI doses, notably the olfactory bulb, striatum, globus pallidus, anterior cingulate cortex, somatosensory cortex, and thalamus, all linked to sensory processing and psychedelic effects. Pretreatment with the 5HT2a receptor antagonist ketanserin completely blocked the positive BOLD response to the 3.0 mg/kg dose, indicating the involvement of 5HT2a receptors. When these receptors were blocked, PSI caused reduced or negative BOLD signals in certain regions. Significant activation was observed in the claustrum, cortico-basal ganglia-thalamic-cortical loop, and cerebellum, revealing the engagement of key circuits in PSI-induced responses. These findings were visualized through 2D statistical heat maps and 3D reconstructions, emphasizing PSI's dose-dependent impact on brain activity. At the 0.3 mg/kg PSI dose, females exhibited significantly greater brain activation than males, with 45 brain regions showing increased sensitivity. Key areas include the prefrontal cortex (prelimbic, ventral orbital, lateral orbital cortices), ventral striatum (accumbens core, shell, ventral pallidum), thalamus, ventral tegmental area, cerebellum, and regions involved in arousal and consciousness, such as the reticular activating system, raphe nuclei, and periaqueductal gray.







BOLD Resting State Functional MRI



Functional connectivity magnetic resonance imaging (fcMRI) illustrated a dose-dependent difference in connectivity among 11 major brain regions, represented as violin plots for all 173 brain regions. The average degree of connections increased with each dose: vehicle (Avg Degree: 10.08), 0.03 mg/kg (22.97), 0.3 mg/kg (22.42), and 3.0 mg/kg (25.47). A significant treatment effect was observed (one-way ANOVA, $p<0.0001$), with many regions like the olfactory system and somatosensory cortex showing greater connections than the vehicle but not among themselves. Notably, the medulla and cerebellum displayed a U-shaped response, with the 0.3 mg/kg dose yielding the lowest connections. The cerebellar nuclei exhibited significant increases in connections with low-dose PSI treatment, expanding to various brain areas, as detailed in a 3D reconstruction.

Future Directions

Exploring additional research avenues can deepen our understanding of psilocybin's effects. One area to investigate is the impact of a normal light-dark cycle, which may reveal circadian influences on brain activity. Additionally, examining brain and plasma lipidomics could provide insights into changes in the endocannabinoid system. Given the cerebellum's unexpected involvement in psilocybin's effects, further studies should explore its role in modulating cognitive, emotional, and sensory processes. The lab plans to investigate psilocybin's long-term effects on neuroplasticity, synaptic changes, and reward and fear processing, particularly concerning degenerative neurological diseases like Parkinson's.

Acknowledgement

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