Tripping Mice and Stoned Fish: Head Twitch Response (HTR) and Behavioral Phenotypic Evidence of Effect Differences Between Synthetic Psilocybin and Psychedelic Mushroom Extract

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BACKGROUND

- Anecdotal reports suggest that the behavioral and pharmacological effects of psilocybin-containing, "*full-spectrum*" psychedelic mushroom extract (FSME) differ from those of chemical psilocybin (PSIL) in their nature and intensity.
- Furthermore, psychedelic mushrooms contain intermediate products of the psilocybin biosynthetic pathway such as baeocystin, norbaeocystin, and aeruginascin that may influence the nature of the effect of psilocybin ("*entourage effect*") along with other components such as harmines with monoamine oxidase inhibiting properties.
- We compared the effect of PSIL to that of FSME on the mouse head twitch response (HTR), which is correlated with psychedelic effects in humans and on a rodent antidepressant screening test (tail suspension test – TST).
- We also compared the effects of PSIL and FSME in a behavioral phenotypic zebrafish model.

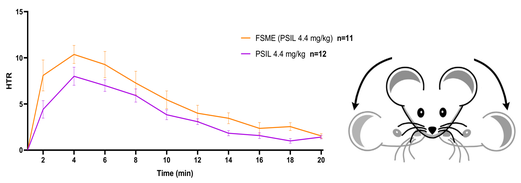


Figure 1A: Effect of 4.4 mg/kg psilocybin and FSME (4.4 mg psilocybin/kg) on HTR over 20 minutes. Three-way time *vs*. treatment interaction p < 0.0001, FSME *vs*. PSIL, p < 0.04.

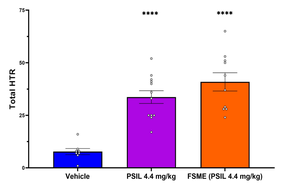


Figure 1B: Effect of 4.4 mg/kg of psilocybin and FSME (4.4 mg psilocybin/kg) on total HTR over 20.

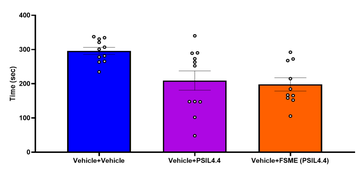


Figure 2A: Effect of psilocybin 4.4 mg/kg and FSME (4.4 mg psilocybin/kg) on **inactive time** in the TST, 2 days after treatment.

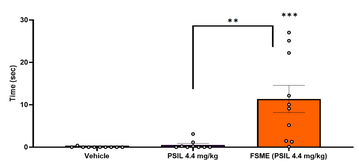
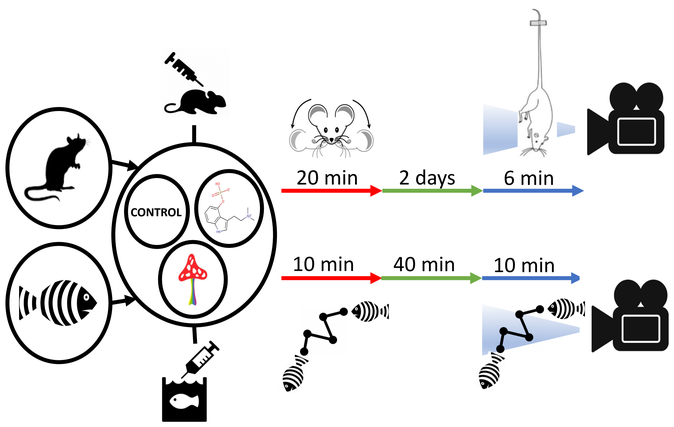
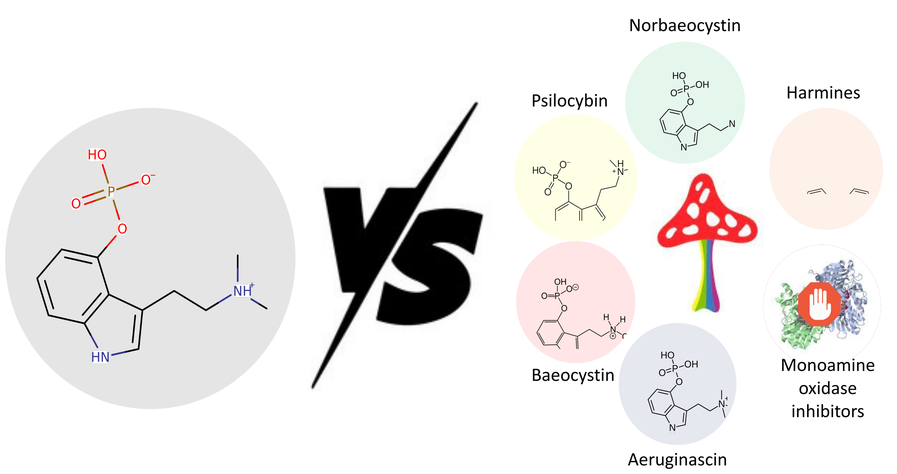


Figure 2B: Effect of psilocybin 4.4 mg/kg and FSME (4.4 mg psilocybin/kg) on **highly active time** in the TST, 2 days after treatment.

****p < 0.0001, ***p < 0.001, **p < 0.01 and *p < 0.1 *vs*. vehicle.





METHODS

- Male C57Bl/6j mice were used in all head twitch and TST studies.
- PSIL (98.75% purity) was provided by Usona Institute (USA). FSME, a methanol extract of *Psilocybe cubensis* (psilocybin content 1.5%) was produced by BYAS-PEB. Drug doses were calculated so that equal injection volumes of PSIL and FSME contained equal concentrations of psilocybin on a mg per kg basis.
- HTR was measured over 20 minutes in a magnetometer-based system using ear clip magnets.
- The TST was conducted using a Noldus EthoVision system 48 hours after drug administration.
- Individual male zebrafish (*Danio rerio*) were used in an open arena, behavioral phenotyping experiment. The drug dose, 3 mg/L of PSIL and FSME containing an equal concentration of psilocybin, was administered in a beaker containing 200 mL of water for 10 minutes. Control fish were placed in a beaker containing 200 mL of water for 10 minutes. The fish were then placed in a 50 x 50 x 4 cm arena and video-tracked and analyzed by Noldus EthoVision XT software, with trajectories recorded for 10 minutes immediately and 60 minutes after treatment.

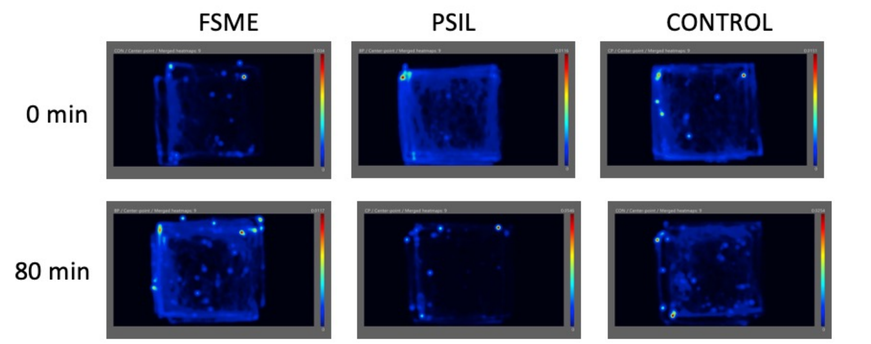


Figure 3A: Heatmap visualization of zebrafish movement for PSIL and FSME *vs*. control groups.

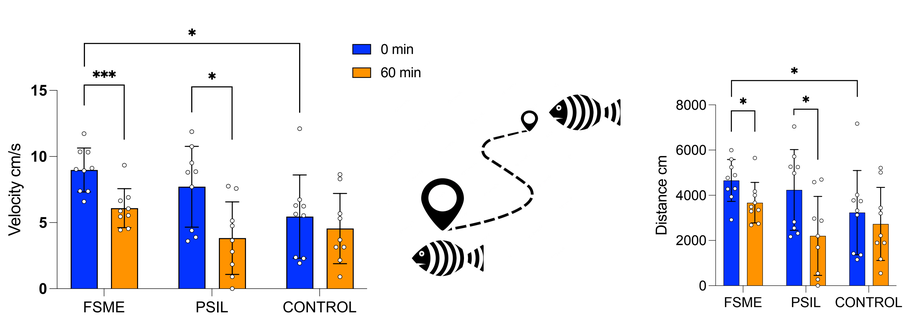


Figure 3B: Zebrafish velocity and distance traveled for psilocybin and FSME *vs*. control groups.

***p < 0.001, **p < 0.01 and *p < 0.1 *vs*. control.

RESULTS AND DISCUSSION

FSME induced a significantly greater number of head twitches over 20 minutes at a psilocybin dose of 4.4 mg/kg than PSIL at the same dose (F=4.41, df 1,21, p=0.04; FSME n=11, PSIL n=12) (**Fig 1A**). On the TST, both PSIL (209.3±93.6 sec, n=11, p=0.01) and FSME (198.14± 61.3 sec, n=10, p=0.0006) showed significantly less inactivity than VEH (296.0±33.8 sec, n=11) (**Fig 2A**). On the highly active measure of the TST, FSME (11.38± 10.13 sec, n=10) induced significantly more activity than both VEH (0.04 ± 0.12, n=11, p=0.0007) and PSIL (0.53±1.04 sec, n=11, p=0.001) (**Fig. 2B**).

In the zebrafish experiment, during the first 10-minute recording period, 2D spatiotemporal reconstructions of the zebrafish swim paths demonstrated clearly visible differences in swimming patterns including velocity, distance swum, average distance to perimeter and middle and changes in direction, between the control and the FSME/PSIL groups (**Fig. 3A**). There were also a number of clear differences in swimming patterns between the FSME and PSIL groups, especially related to the mean time spent in the corners of the arena. At 60 minutes, the swimming pattern of the PSIL group closely resembled that of the control group, whereas the FSME group continued to show a similar, slightly attenuated swimming pattern to that observed in the initial 10-minute recording period **(Fig. 3B).**

CONCLUSIONS

A prior study by Zhuk *et al*. [1] suggested that mushroom extract has greater potency in inducing HTR than psilocin (the active metabolite of psilocybin). Our findings in mice are in accordance with this observation. We have further shown that on the TST, a screening test for antidepressant potential, FSME induces a stronger effect than PSIL when the same dose of psilocybin is administered with both preparations. Furthermore, this work provides evidence of a robust and measurable zebrafish response to PSIL and FSME. The more sustained effect of FSME may be indicative of an "*entourage effect*". Further studies are indicated to elucidate a possible therapeutic advantages of "*full-spectrum*" psychedelic mushroom extract as compared to chemical psilocybin and to identify the entourage molecules that contribute to this effect.

REFERENCES

[1] Zhuk, Olga, *et al.* (2015) Research on acute toxicity and the behavioural effects of methanolic extract from psilocybin mushrooms and psilocin in mice. Toxins. 7 (4): 1018 - 1029.

ACKNOWLEDGEMENTS

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