CHARACTERISATION OF BEHAVIOURAL, SAFETY AND PHARMACOKINETICS (PK) PROPERTIES OF PSILOCYBIN AND PSILOCIN IN RODENTS

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Introduction

Psychedelic drugs, e.g. psilocybin (PSY) are becoming widely recognized for their therapeutic potential to treat psychiatric conditions including depression and addictive disorders. PSY is rapidly metabolised to psilocin (PSI) which underlies its primary pharmacological properties. Despite PSY being evaluated in Phase 2/3 clinical trials, relatively little preclinical data for PSY exists to address issues such as PK/PD relationship between behaviours associated with CNS 5-HT_{2A}R engagement, e.g head twitch (HT; mouse; Corne and Pickering, 1967) and wet dog shakes/back muscle contractions (WDS/BMC; rat) as well as other behaviours attributable to non-5-HT_{2A}R (see Halberzettl et al, 2013). Also, the relationship between these behaviours and the discriminative stimulus effects of PSY are relatively unexplored. The present series of experiments were designed to evaluate some of these issues, in part for the purpose of benchmarking newer 5-HT_{2A}R agonists currently under investigation. In the present study we have included the Mindset NCE MSP-1014 as one such example.

- Primary objectives
  - Characterise psilocybin (PSY) effect of cardinal 5-HT signs (2A: HT/WDS/BMC; 2C: PG; 1A: HLA/FPT*)
  - Establish plasma levels of PSY and psilocin (PSI) at doses that induce cardinal 5-HT behavioural signs.
  - Evaluate effects of PSY on motor activity (LMA) and core body temperature (CBT), i.e side effects.
  - Evaluate plasma:CSF relationship of PSI in the rat.
  - Establish PSY discriminative cue to evaluate PSY oral vs. SC potency and duration of action.
  - Benchmarking purpose for evaluating novel psychedelic drugs – MSP-1014 presented as example.

* HT = head twitch (mouse); WDS = wet dog shakes (rat); BMC = back muscle contractions (rat); PG = penile grooming; HLA = hind limb abduction; FPT = forepaw treading

Methods

Mouse head twitch:. Male, C57BL/6J mice (body weight range 20-30g) were dosed with the appropriate dose of test article, and following a 1-minute pre-treatment time, placed in individual observation chambers. Animals were visually assessed for the incidence head twitches (HT) continuously over a 1hr period. Head twitches were defined as a rapid jerk of the head which was not elicited by an external tactile stimulus (Corne and Pickering, 1967). Each head twitch was individually counted by a trained observer, and the data expressed as the mean+SEM of 6-10 mice per group. Mice were study naïve prior to test. Head twitch experiments were conducted at both IVS and TPH laboratories. Care was taken to align methods between both sites to the extent possible.

Mouse locomotor activity:. Following a minimum washout period of 7 days after HT, C57BL/6J mice were dosed with test article or vehicle, and placed in individual observation chambers (dimensions 17" W x 17" L x 12" H) for the recording of distance travelled and rearing counts over a 1h period.

Mouse body temperature:. Following a minimum washout period of 7 days after HT, rectal body temperature of male C57BL/6J mice was assessed immediately prior treatment with test article or vehicle, and at 1h and 2h post treatment. Data expressed as the mean difference score between pre- and 1h and 2h post-treatment.

Rat behavioural test: Male, Sprague-Dawley rats (body weight range 250-400g) were dosed with the appropriate dose of test article and following a 1-minute pre-treatment time, placed in locomotor activity boxes (dimensions 17" W x 17" L x 12" H) and continuously monitored for a 1 hr period with data collected into 10 minute time bins. Animals were visually assessed for overt behavioural signs, including behaviours characteristic of 5-HT_{2A} receptor activation (wet dog shakes, back muscle contractions), 5-HT_{2A} receptor activation (yawning, penile grooming) and 5-HT_{1A} behaviours (forepaw treading, hindlimb abduction) (see Halberzettl et al, 2013). Additional behavioural and somatic signs characteristic of 5-HT syndrome (e.g. tremor, salivation, flat body posture, core body temperature change) were also measured. Simultaneously, the spontaneous activity of the rats was measured using an automated tracking system (Med Associates, VT, USA). Activity data collected included total distance traveled, rearing counts and ambulatory episodes. All data were expressed as the mean+SEM of 6-10 rats per group.

Psilocybin drug discrimination: Male Sprague-Dawley rats were initially food restricted by presentation of 18-20g food at day end (single housing). After 7 days acclimatisation to the food restriction procedure, they were trained daily to lever press for food (45mg Bioserve pellet) in standard 2-lever operant conditioning chambers controlled by Med-PC software over a period of 1 week (Med. Associates Ins., St. Albans, VT). The rats were trained to lever press for food to an FR10 value (i.e 10 lever presses for a single food reward). Once stable food responding was acquired to both response levers, discrimination training began. Over a period of 20-50 training sessions, the rats were trained to associate one lever to a psilocybin training dose of 0.5 mg/kg SC, and the second lever to a neutral stimulus (saline, SC) (Winter et al, 2007). Training sessions lasted 30-min or until the delivery of 50 pellets and continued until the animals attained appropriate stimulus control (defined as six consecutive sessions where animals made no more than 16 lever presses before the delivery of the first reward, and at least 95% total responses on the appropriate lever). The rats continued to receive daily food ration in their home cage at day end.

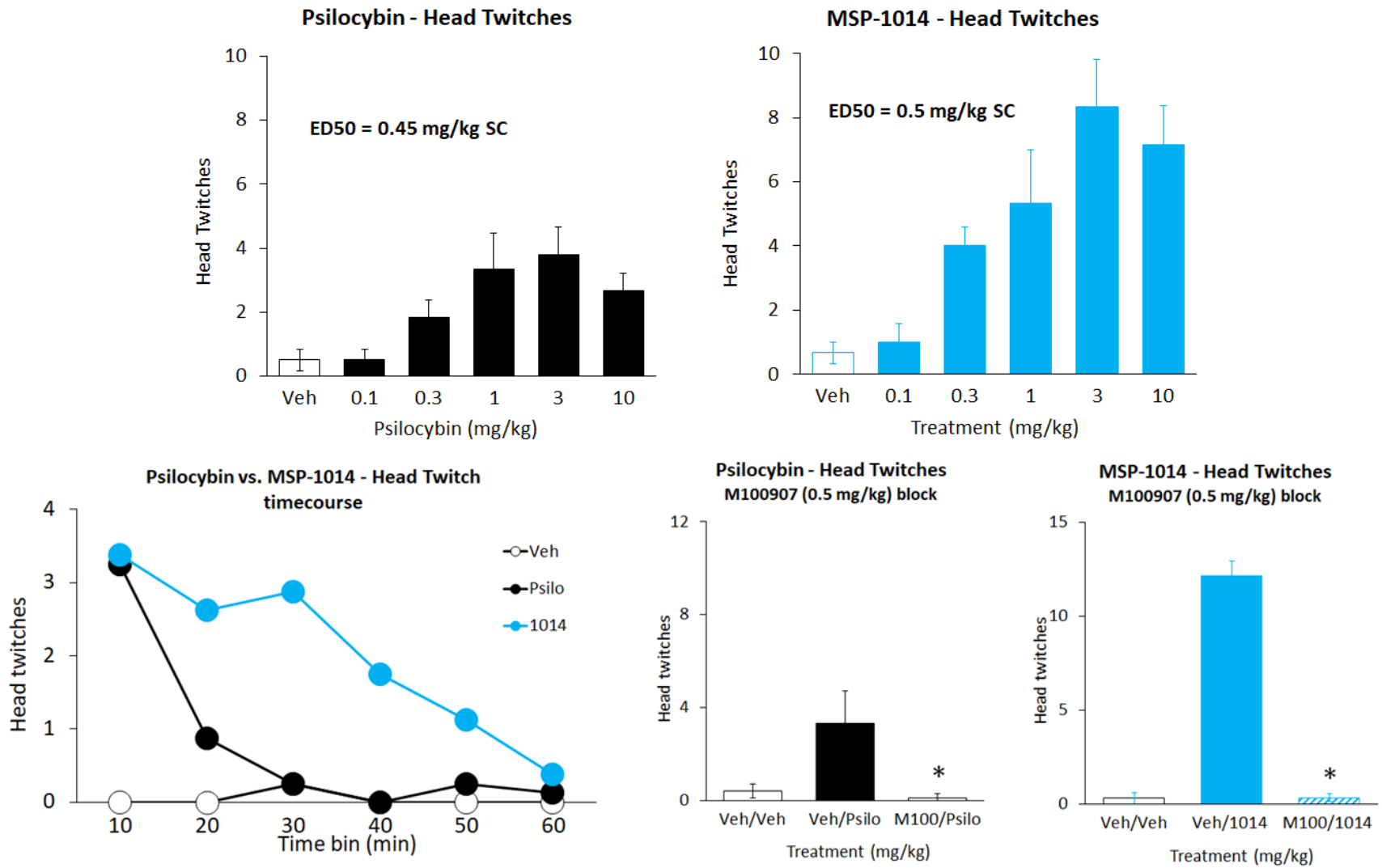
Once trained, tests of substitution (drug treatment by either SC or oral route of administration) or duration of action (oral route of administration) were conducted. On test days, both levers were designated active, i.e., every 10th response on either lever resulted in delivery of a food pellet. Test sessions continued until 50 pellets had been obtained or 30 min had elapsed. During these sessions response rate was also measured.

Rat PK and CSF sampling: Male, Sprague-Dawley rats were used. Catheters were implanted in the carotid artery (CAC) for serial blood collection and the cisterna magna (CMC) for serial cerebrospinal fluid (CSF) collection. Following test article administration, serial blood samples were collected from the CAC and the sample volume replaced with saline. Serial CSF samples were collected from the CMC concurrently to the plasma sample collection. Blood samples were transferred into K_2EDTA tubes on wet ice and centrifuged within 10 min (3200 x g for 5 min at 4°C) to obtain plasma. Samples were stored frozen at –80°C until bioanalysis.

Mouse PK: Male, C57BL/6J mice were used. Serial blood samples were collected via tail snip following drug administration. Blood samples were processed to plasma as stated above. Samples were stored frozen at –80°C until bioanalysis.

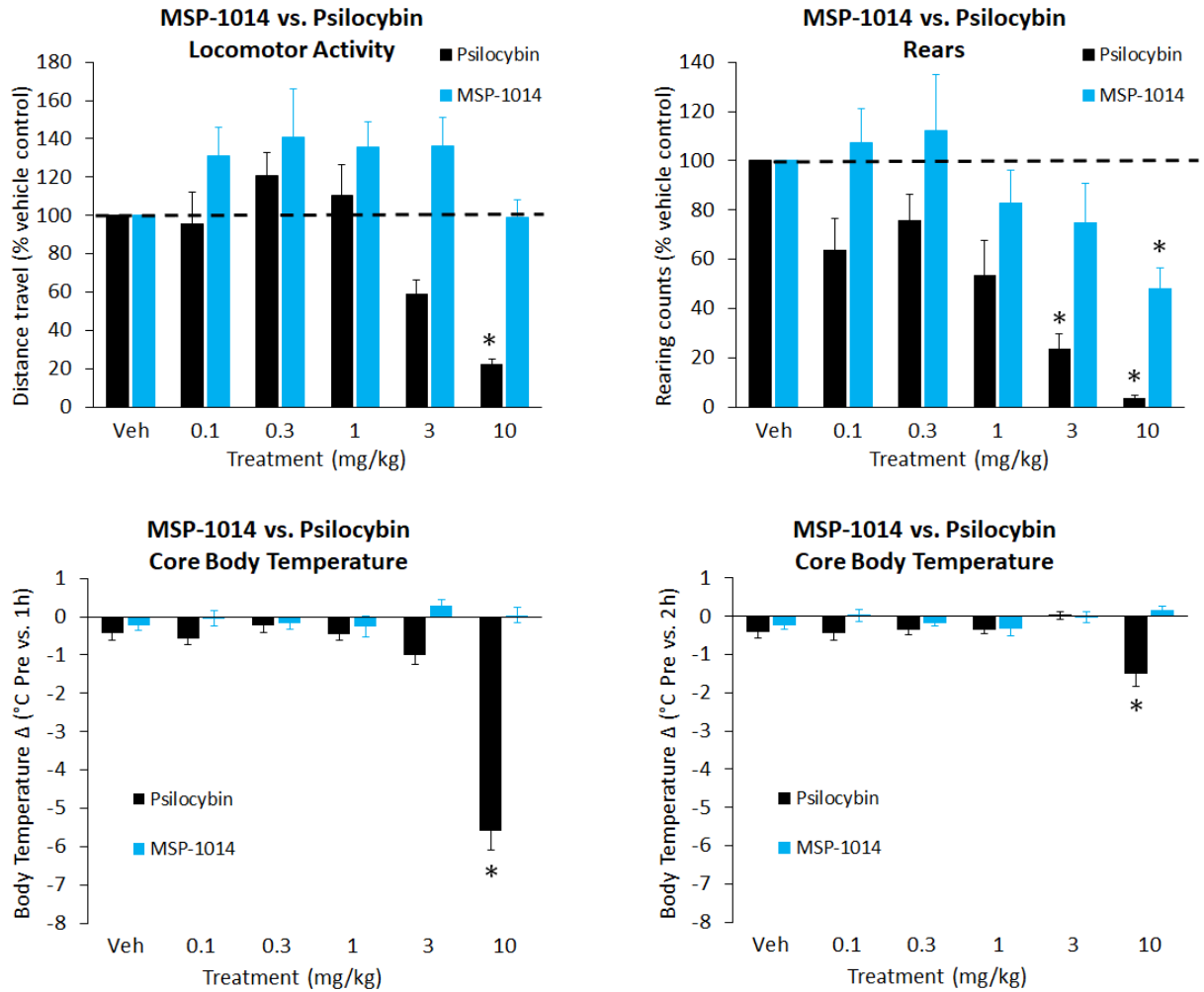
Bioanalysis:. Samples were analyzed by an AB Sciex API4000 QTRAP LC-MS/MS system equipped with an ESI source in positive ion mode. Plasma samples were extracted with 50/50 methanol/acetonitrile containing internal standard (psilocin-d10) and separated on two serially connected Javelin Aquasil C18 columns (2.1 × 20 mm, 5 µm each; Psilocin). CSF samples were diluted two-fold with 2 mM ammonium acetate in acetonitrile and analyzed as above.

Comparison between Psilocybin and MSP-1014 in the mouse



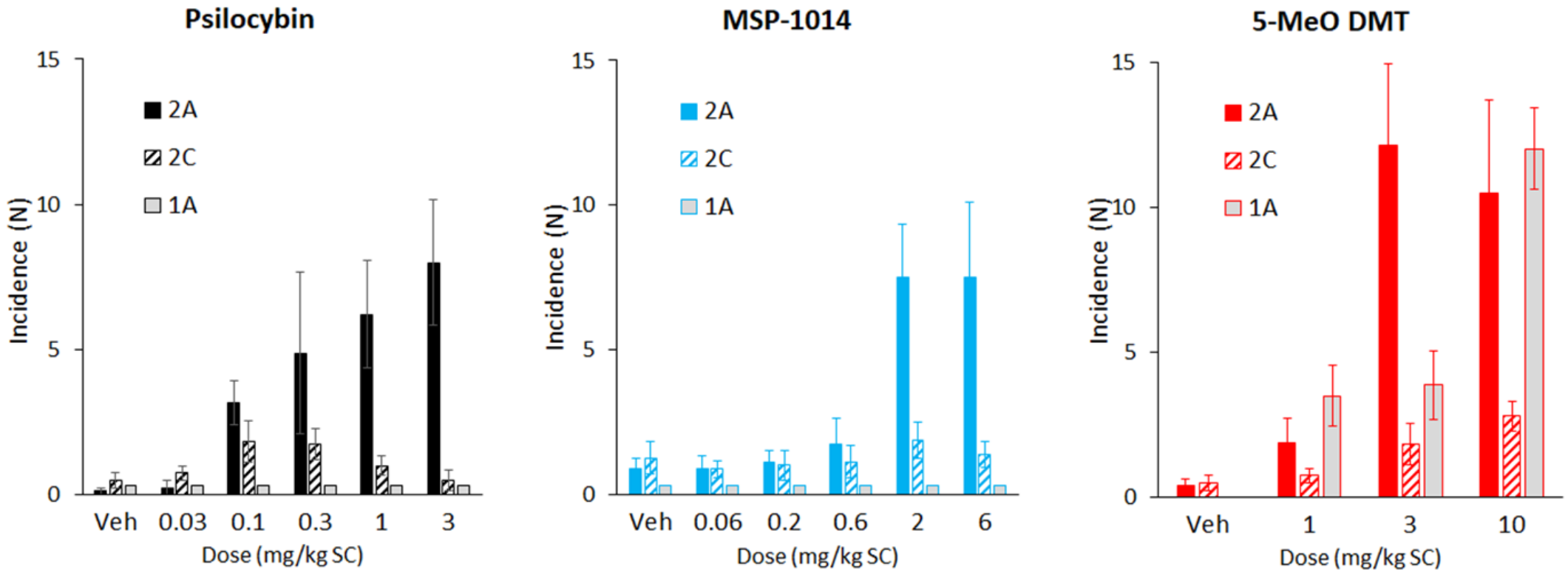
(A) Both Psilocybin (0.1-10 mg/kg SC) and MSP-1014 (0.1-10 mg/kg SC) elicited a dose related incidence of head twitches in male C57BL/6J mice. The magnitude of head twitch response seemed higher in MSP-1014 treated mice and this was confirmed in a second experiment **(B)** where the effect of psilocybin (3 mg/kg SC) and an equimolar dose of MSP-1014 (6.1 mg/kg SC) were directly compared. The increase in head twitch response induced by MSP-1014 was the result of an extended duration of action. In a further experiment **(C)**, head twitches induced by either psilocybin (3 mg/kg) or MSP-1014 (6.1 mg/kg SC) were blocked by the selective 5-HT2A antagonist M100907 (0.5 mg/kg IP).

Comparison between Psilocybin and MSP-1014 in the mouse



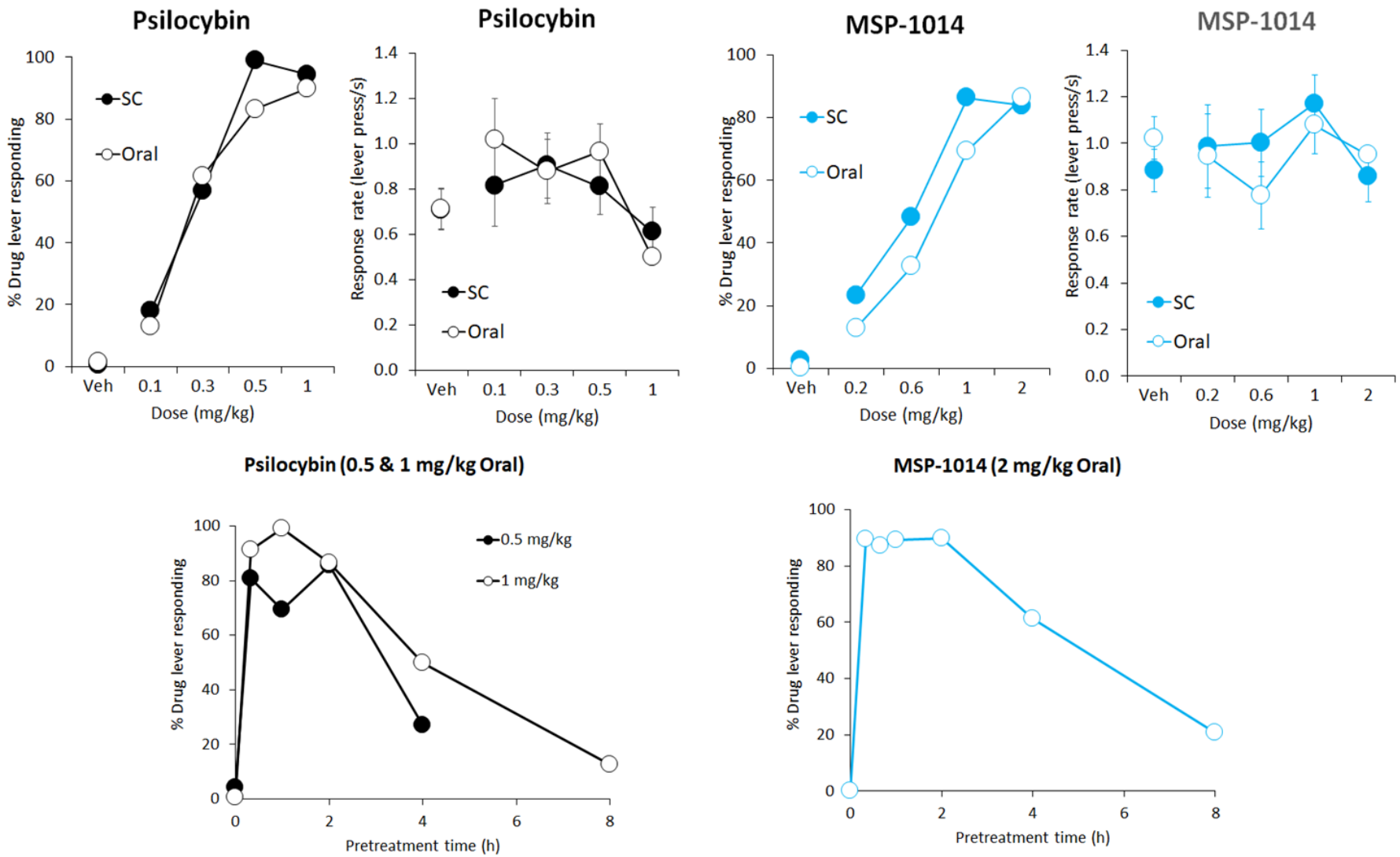
(A) Psilocybin (0.1-10 mg/kg SC) produced a dose related decrease in locomotor activity measured as distance travelled and rearing counts in a 1h test conducted in in male C57BL/6J mice. In contrast, equivalent doses of MSP-1014 (0.1-10 mg/kg SC) elicited a milder effect on locomotor activity with only a significant decrease in rearing counts relative to vehicle pretreatment at the 10 mg/kg dose. For the purpose of comparison, since both psilocybin and MSP-1014 were run in sep[arate study cohorts with differing baseline activity levels, the effect of each drug are presented as a % of vehicle baseline. **(B)** Psilocybin (10 mg/kg SC) produced a significant hypothermia relative to vehicle pretreatment. An equivalent dose of MSP-1014 had no effect on core body temperature. All data presented as means+SEM. N=6-8 per group. * P<0.05 vs. vehicle pretreatment (Dunnetts test following ANOVA).

Comparison between Psilocybin and MSP-1014 in the rat: overt behaviour



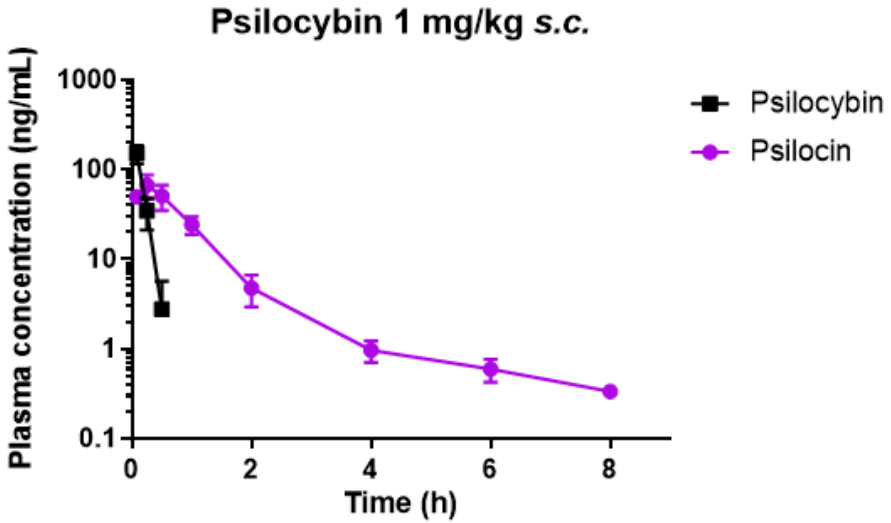
(A) Both Psilocybin (0.03-3 mg/kg SC) and MSP-1014 (0.06-6 mg/kg SC) elicited a dose related incidence of WDS and BMC, i.e cardinal 5-HT2AR behavioural signs. The magnitude of these effects were similar between both drugs. Only occasional signs characteristic of 5-HT2CR (PG) or 5-HT1AR (FPT, HLA) were seen across this dose range. No reliable effects of either drug on locomotor activity or core body temperature were noted at these doses. In contrast, 5-MeO DMT (1-10 mg/kg SC) elicited a dose related incidence of WDS/BMC and cardinal 5-HT1AR signs of FPT/HLA. A significant decrease in rearing counts and core body temperature was also noted (data not shown).

Comparison between Psilocybin and MSP-1014 in the rat: drug discrimination



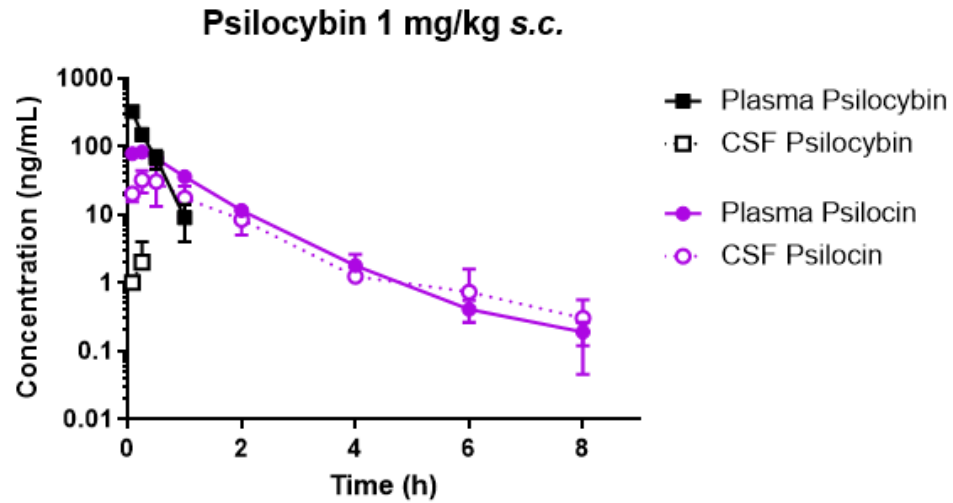
(A) Psilocybin (0.1-1 mg/kg) produced dose related generalisation to a psilocybin (0.5 mg/kg SC) cue. Psilocybin was equipotent following either SC (10min ptt) or oral (20min ptt). Response rate was not significantly affected but showed a trend to decline at doses >1 1mg/kg. MSP-1014 (0.2-2 mg/kg) also showed a dose-related generalization to a psilocybin-cue following either SC (30min ptt) or oral (40min ptt) administration. Response rate was unaffected across this dose range. Both psilocybin (1 mg/kg) and MSP-1014 (2 mg/kg) showed a similar duration of action with an approximate active half-life of 4h. (N=7-10 rats per treatment group).

Psilocybin (1 mg/kg SC) PK studies in mouse and rat



Parameter	Parameter estimate (Mean ± SD)	
	Psilocybin	Psilocin
t_{max} (h)	0.0833 ± 0.00	0.250 ± 0.00
C_{max} (ng/mL)	150 ± 35.4	66.7 ± 20.0
Apparent $t_{1/2}$ (h)	nc[a]	1.71 ± 0.317
$AUC_{0\text{-tlast}}$ (h*ng/mL)	22.3 ± 6.35	63.2 ± 12.5
F (%)	16.2 ± 4.60	-

[a] nc denotes not calculable as the terminal phase is not well defined.



Parameter	Parameter estimate for Psilocin (Mean ± SD)	
	Plasma	CSF
t_{max} (h)	0.250 ± 0.00	0.278 ± 0.210
C_{max} (ng/mL)	82.6 ± 13.3	35.1 ± 10.9
Apparent $t_{1/2}$ (h)	1.23 ± 0.180	2.45 ± 0.819
$AUC_{0\text{-tlast}}$ (h*ng/mL)	94.4 ± 0.450	47.4 ± 22.3
$AUC_{0\text{-tlast}}$ CSF/plasma ratio	0.502 ± 0.236	

Comparison between head twitch studies conducted at IVS and TPH

	IVS		TPH	
	ED_{50}	E_{max}	ED_{50}	E_{max}
Psilocybin	0.45 (0.13-1.8)	5±2	0.18 (0.10-0.34)	31±2
LSD	0.06 (0.03-0.14)	6±2	0.11 (0.02-1.0)	36±3
5-MeO DMT	8.0 (2.8-26.6)	12±5	NT	NT
DOI	NT	NT	0.70 (0.3-18.4)	115±10

Comparison of the number of HT at maximal dose (i.e Emax) and the calculated ED50 for psilocybin and LSD following studies conducted at IVS and TPH. Note that despite attempts to standardize methods between sites and overlapping ED50's, there is a large disparity in the number of HT's recoded, with scores significantly higher in the TPH experiments. Data analysed by Prism software. ED50 levels in mg/kg SC with 95% confidence levels. Emax is a mean+SEM for the highest number of HT's recoded by a single dose.

Summary and conclusions:

1. PSY-induced HT were restricted to 10-20min post dose and unrelated to PSI plasma half-life. HT incidence appeared to be reduced by emergence of hypolocomotion/hypothermia.
2. Plasma levels (C_{max}) of PSI at approx. ED_{50} for induction of HT (mouse) and WDS (rat) show equivalence to clinical exposures associated with psychedelic effect (~20 ng/ml; e.g. Madsen et al (2019).
3. PSY had equivalent SC and oral potency based on generalization to a PSY (0.5 mg/kg SC) cue. PSY (1 mg/kg oral) had an active half-life of ~4 h in this assay.
4. PSI appeared rapidly in mouse plasma and rat plasma and CSF (t_{max} ~0.25 h) following PSY dosing (1 mg/kg SC). Plasma exposure (C_{max} and AUC) of PSI was similar in magnitude between mouse and rat. The rat CSF/plasma AUC ratio (partition coefficient, Kp) for PSI was 0.5 and its half-life ($t_{1/2}$) in CSF appeared to be ~2-fold longer than that in plasma. Taking into account the % PSI unbound in rat plasma (85.4%, data not shown), the CSF/plasma unbound Kp (Kp,uu) is estimated as 0.59.
5. MSP-1014 induced robust 5-HT_{2A}R mediated behaviours in both mouse and rat, with a similar overall potency to PSY. However MSP-1014 at the dose range tested, was devoid of the hypolocomotion/hypothermia effects noted following PSY pretreatment.
6. MSP-1014 generalized fully to a PSY cue following both SC and oral pretreatment with a duration of action similar to PSY.
7. While mouse HT studies conducted between IVS and TPH showed similar ED_{50} values, the magnitude of HT response measured at TPH labs was significantly higher.

References:
Corne SJ, Pickering RW (1967) Psychopharmacologia 11(1): 65-78; Haberzettl R, Bert B, Fink H, Fox MA. (2013) Behav Brain Res. 256: 328-345.
Madsen et al (2019) Neuropsychopharmacol. 44: 1328–1334; Winter JC, Rice KC, Amorosi DJ, Rabin RA. (2007) Pharmacol Biochem Behav. 87(4): 472-480.