Psilocin and ketamine microdosing: effects of subchronic intermittent microdoses in the elevated plus-maze in male Wistar rats

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Short-term moderate doses of serotonergic and dissociative hallucinogens can be useful in the treatment of anxiety. Recently, a trend has developed for long-term intermittent 'microdosing' (usually one-tenth of a 'full' active dose), with reports of long-lasting relief from anxiety and related disorders; however, there is no scientific evidence for the efficacy of therapeutic microdosing nor to show its lasting effects. The objective of this study was to test for lasting effects on anxiety in rats after microdosing with ketamine or psilocin. Over 6 days, Wistar rats ($N = 40$) were administered ketamine (0.5 or 3 mg/kg), psilocin (0.05 or 0.075 mg/kg), or saline on three occasions. A 5-min elevated plus-maze test was conducted 48 h after the final drug treatment ($n = 8$). Dependent variables were entries (frequency), spent time (%), and distance traveled (cm) in each zone, as well as total frequency of rears, stretch-attend postures, and head dips. Statistical analyses of drug effects used separate independent one-way analysis of variance and pair-wise comparisons using independent *t*-tests. Statistical effects were modest or borderline and were most consistent with a mildly anxiogenic profile, which was significant at lower doses; however, this conclusion remains tentative. The lower doses of ketamine and psilocin produced comparable effects (to one another) across each variable, as did the higher doses. This pattern of effects may suggest a common (e.g. neurotransmitter/receptor) mechanism. We conclude that microdosing with hallucinogens for therapeutic purposes might be counter-productive; however, more research is needed to confirm our findings and to establish their translational relevance to clinical 'psychedelic' therapy. *Behavioural Pharmacology* 29:530–536

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Introduction

Ketamine (a dissociative anesthetic with psychedelic effects at subanaesthetic doses) is primarily a noncompetitive *N*-methyl D-aspartate (NMDA) receptor antagonist; however, it has also substantial affinity for the serotonin [5-hydroxytryptamine (5-HT)] transporter (SERT) as well as additional effects on dopamine (DA) and opioid neurotransmission (White and Ryan, 1996; Vollenweider, 2001; Mion and Villevieille 2013). Psilocin and its phosphorylated ester psilocybin (which is contained in psychedelic 'magic' mushrooms; Hasler *et al.*, 1997; Passie *et al.*, 2002; Tylš *et al.*, 2014) act as competitive agonists at 5-HT receptors, particularly 5-HT_{1A} and 5-$HT_{2A/C}$ (Nichols, 2004), which are widely distributed in the neocortex, basal ganglia, limbic system, hippocampus, and in the case of 5-HT_{1A}, in raphe nuclei (Barnes and Sharp, 1999; also see Tylš *et al.*, 2014). Actions at DA D2 receptors have also been implicated; for example, in humans, psilocybin and ketamine both resulted in increased extracellular DA concentrations in the ventral striatum which correlated with depersonalization (Vollenweider *et al.*, 1999, 2000), however, DA-ergic mechanisms are debated (Vollenweider, 2001; Ray, 2010).

Although psychedelic hallucinogens and dissociative anaesthetics possess distinct pharmacological properties, it has been argued that they exert some of their psychomimetic effects [e.g. disruption of prepulse inhibition (PPI)] through a common pathway. Vollenweider (2001) proposed distributed cortico-striato-thalamic 5-HT_{2A} receptors as a central substrate of psychomimesis, although he also points out that psychedelic dissociative anaesthetics and 5-HT-ergic hallucinogens operate by glutamatergic non-NMDA receptors in frontocortical systems (Moghaddam and Adams, 1998; Aghajanian and Marek, 2000). Consistent with a shared mechanism, moderate doses of ketamine and psilocybin/psilocin exert similar psychomimetic behavioral effects in rodent models of psychosis such as impairment of sensory-motor gating in PPI (Swerdlow and Geyer, 1998; Geyer and Swerdlow, 1999; Geyer *et al.*, 2001; Geyer and Vollenweider, 2008; Páleníček *et al.*, 2011; Tylš *et al.*, 2016). In likewise, in humans, these drugs can result in acute psychomimetic responses that are reminiscent of psychosis with symptoms including hallucinations, thought disturbances, and cognitive disorganization (Adams and Werner 1997; Tylš *et al.*, 2016) as well as

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anxiety and panic attacks (Bluelight, 2017; Shroomery, 2017). In consequence, research for many decades has oriented to psychedelic hallucinogens and dissociative anaesthetics as deleterious to mental health.

More contemporary research has suggested that psilocybin and ketamine can have therapeutic properties when administered at moderate psychomimetic doses (Moreno *et al.*, 2006; Grob *et al.*, 2011; Bogenschutz and Pommy, 2012; Ibrahim *et al.*, 2012; Zarate *et al.*, 2012; Irwin *et al.*, 2013; Parise *et al.*, 2013; Stebelska, 2013; dos Santos, 2014). Lapidus *et al.* (2014) showed that two daily doses of intranasal ketamine (50 mg) had antidepressant properties, while producing minimal subjective psychedelic effects. The lack of such effects is somewhat surprising, as for insufflation, 50 mg is considered a 'common' or medium dose, with 60 mg and more considered 'strong' (Erowid, 2017, but also see howtousepsychedelics.org, 2017). Related to this, a recent trend has been developing for what has become known as 'microdosing' with drugs such as psilocybin, lysergic acid, ketamine, and methoxetamine (Erowid, 2017; Reddit, 2017; The Third Wave, 2017). This involves self-administering 'microdoses' (approximately one-tenth of a full dose, as defined by Fadiman, 2011) on an intermittent regime, which can vary from once or twice per week or month (but not exceeding alternate days). Fadiman (2011) recommends dosing every third day. With sublingual ketamine, it is recommended that users should titrate up (as required) from starting doses 'far below' 50 mg (~0.75 mg/kg), with effectiveness probable at doses below 0.3 mg/kg, and for oral ketamine, doses are closer to 50–100 mg (howtousepsychedelics.org, 2017, also see Reddit, 2017). With psilocybin magic mushrooms, the regime is the same, but the recommended oral dose is 0.2–0.5 g (howtousepsychedelics.org, 2017; The Third Wave, 2017). The doses are specifically intended to be 'sub-perceptual', that is, without noticeable psychedelic effects, and users claim rapid (from 2 h) relief from anxiety and depression that can last for several days, even weeks or months (howtousepsychedelics.org, 2017; The Third Wave, 2017). As well as apparently treating psychological ill-health, it has become very fashionable to use microdosing to enhance creativity, spirituality, and social relationships (The Third Wave, 2017).

Currently, evidence for therapeutic microdosing is purely anecdotal (reported on drug discussion fora and blogs online, and in Fadiman's, 2011 book). To date, there is no published scientific research (in humans or rodents) that examines effects of microdosing with serotonergic or dissociative psychedelics or any attempt to establish a preclinical model of microdosing. Such a model would result in possibilities for microdosing drug testing and development, as well as for understanding the neural mechanisms that may be involved. Unsurprisingly, the commercialisation of microdosing has already begun, with 'kits' and accessories available to purchase (MicroKits: *https://microkits.co.uk/collections/all*). In the absence of any evidence that microdosing is effective, consumers may be wasting their money, and although adverse effects seem unlikely, they have not yet been discounted.

The present study aimed to test, in rats, the hypothesis that subchronic, intermittent microdoses of subcutaneous ketamine (0.5 and 3 mg/kg) and psilocin (0.05 and 0.075 mg/kg) would result in anxiolytic/genic effects (compared with saline) when tested 'drug-free' on the elevated plus-maze (EPM), which is a widely used preclinical pharmacological screen for anxiolytic drugs.

Methods

Design

The ketamine and psilocin data were analyzed separately. In each case, an experimental design was used with drug (low, high, or saline) as a between-subjects factor. The dependent variables were as follows: total arm entries and total distance traveled (cm) within the maze; frequency of entries and time (%) spent within each zone (central square, open arms, and closed arms); and the distance traveled (cm) per visit to open and closed arms. Arm entries were counted when the center of the body crossed into the zone. Scores for (open/closed) arm time on the maze were calculated as percentages of total time spent in the arms, whereas scores for time in the central square were expressed as a percentage of total time in the maze. Frequency of rears, stretch-attend postures, and head dips were also recorded. Rears were defined as a vertical upward stretching motion, with front limbs off the floor, placed on the EPM walls for support (no unsupported rears were observed); stretch-attend postures were counted as horizontal, scanning, flat-backed postures; and head dips were defined as the lowering of the rat's snout until level with, or below the platform (Rodgers and Johnson, 1995; Horsley *et al.*, 2007).

Subjects

Forty ($n=8$) naive male Wistar rats (Charles Rivers, Harlow, UK) were caged in groups of four on a 12 : 12 h light/dark cycle with water and standard laboratory diet available *ad libitum* in the home cage. All dosing and testing occurred during the light phase (between 12.00 and 16.00 h). Rats weighed between 292 and 447 g at the time of EPM testing. The data for both drugs were collected concurrently, and so (to minimize animal use) the same saline control animals were used in both analyses. Guidelines of the European Union (86/609/EU) and the National Committee for the Care and Use of Laboratory Animals (Czech Republic) were adhered to, and the research received ethical approval (Reference: MEYSCR-27527/2012-31).

Apparatus

The EPM was of standard size and was made of Perspex with black platform inserts (for contrast with albino rats).

It comprised two opposing open arms (10 cm wide, 50 cm in length, without lips) and two opposing closed arms of the same dimensions but enclosed on three sides by 40 cm high walls. The four arms formed a plus (+) shape, with a 10 cm^2 square central hub. The maze platforms were elevated to a height of 50 cm from the base. Behaviors on the maze were recorded (using a Logitech HD Pro C920 web-camera, Logitech International, Lausanne, Switzerland). Behavioral capture and preprocessing used the video-tracking software, Viewer 3 (Biobserve GmBh, Bonn, Germany).

Procedure

The EPM is sensitive to changes in anxiety (open arm behaviors), and locomotion (closed arm entries) (File, 1992; Pellow *et al.*, 1985; Pellow and File, 1986), as well as changes in exploratory activity, for example, central square behaviors (Cruz *et al.*, 1994; Rodgers and Johnson, 1995). Behavioral procedures were based on the study by File *et al.* (2000) and Horsley *et al.* (2007) and were as follows: after 1 h habituation adjacent to the testing room, rats were taken individually to the testing room, which was dimly lit. The rat was placed on the central square of the maze, facing an open arm (open arm direction alternated between rats) and was allowed to move freely about the maze for 5 min (in the absence of the experimenter), and their behavior was video recorded. Between rats, the maze was wiped clean with tap water. Drug testing order was counterbalanced to distribute the different doses and drug treatments throughout the testing session.

Drugs and doses

Commercially available racemic ketamine solution (Narketan 100 mg/ml; Vetoquinol, s.r.o., Nymburk, Czech Republic) was diluted in physiological saline to a volume of 0.5 or 3 mg/ml for subcutaneous injection at 0.5 or 3 mg/kg in a volume of 1 ml/kg. Psilocin (THC-Pharm GmBh, Frankfurt, Germany) was first added to 200-μl physiological saline (0.9% NaCl) and acidified with 10 μl of glacial acetic acid (30%) and shaken vigorously until dissolved. Physiological saline was then added to dilute to 0.075 or 0.05 mg/ml for subcutaneous injection at 0.075 or 0.05 mg/kg (again, at a volume of 1 ml/kg). Enough drug was made for the whole experiment, which was then stored in the freezer until required. The drug solutions were protected to minimize exposure to light and air during storage and use. Physiological saline was used as a vehicle control and was administered at an equivalent volume per kg weight. Rats received three doses of drug or saline (in the home cage between 12.00 and 16.00 h) over 6 days, with the final dose given on day 6, 48 h before EPM testing (on day 8).

In the open field, PPI, carrousel maze and Morris water maze, psilocin is behaviorally effective in the range of 0.25–4 mg/kg (Páleníček *et al.*, 2011; Rambousek *et al.*, 2014). For our lower dose, we selected 0.05 mg/kg (1% of the highest effective dose tested previously), and our higher dose of 0.075 mg/kg was selected on the basis of unpublished pilot data and was also within the 1–10% range that constitutes a 'microdose'. Across a variety of behavioral tasks (open field, PPI, forced swim test, and the EPM), ketamine is behaviorally effective at 5–50 mg/kg. Our lowest dose, 0.5 mg/kg, is 10% of the lowest and 1% of the highest behaviorally effective doses reported for ketamine. Three mg/kg (rather than 5 mg/kg) was selected as our higher dose as 10% of 50 mg/kg (an anyway unusually high dose for behavioral testing) is 5 mg/kg, which constitutes a 'full' behaviorally effective dose.

Statistical analysis

For each drug, each dependent variable was analyzed using a separate one-way analysis of variance. The α criterion for rejection of the null hypothesis was set at P less than 0.05 (two tailed) unless specifically stated otherwise. Planned comparisons to explore significant main effects used independent t-tests based on the individual error terms. Where Levene's test for equality of variances was significant, corrected statistics are given (adjusted degrees of freedom are rounded to the nearest whole number for presentational purposes).

Results

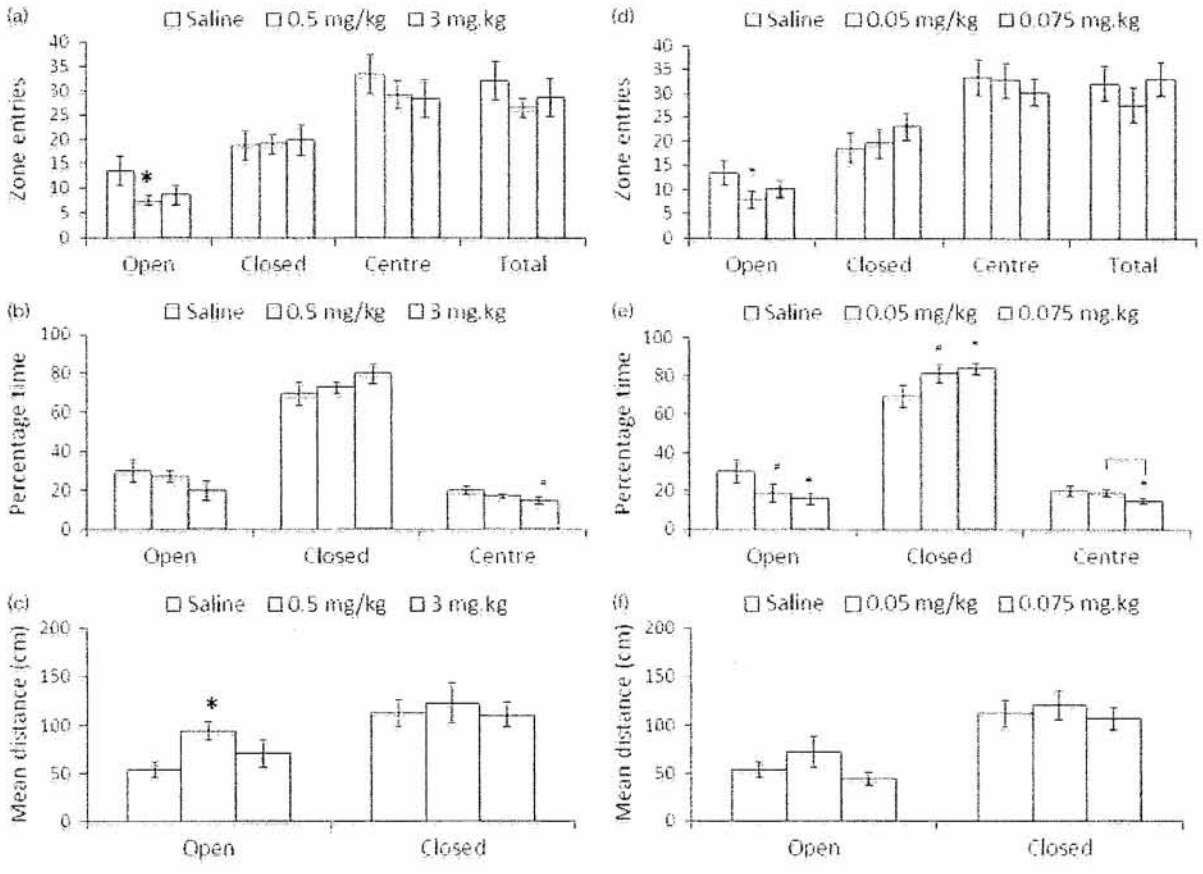
Ketamine

One animal in the 3-mg/kg ketamine group was excluded from all analyses because initial exploration of the data showed that this animal's behavior was aberrant (>2 SDs from the mean) on entries, % time, and distance traveled (ethological behaviors were therefore not scored for this animal).

There was a marginally significant effect of drug treatment on frequency of entries into the open arms [$F(2, 20) = 3.23$, $P = 0.06$; Fig. 1a]. As analysis of variance can be insensitive with smaller experimental group sizes, we followed up this marginal effect; t-tests showed that compared with saline, 0.5 mg/kg significantly reduced the number of visits to the open arms [$t(8) = 2.41$, $P < 0.05$], but 3 mg/kg did not [$t(13) = 1.56$, NS]. Drug treatment did not affect total arm entries, nor did it affect the frequency of entries into the closed arms or center zone [maximum $F(2, 20) = 0.84$, NS].

Means for % time in maze zones suggested that ketamine reduced time in the open arms and center and increased time in the closed arms compared with saline (Fig. 1b); however, statistically these differences were not significant, except for a marginally significant effect on time in the center [$F(2, 20) = 1.96$, $P = 0.08$, one tailed]. Independent t-tests showed that 3 mg/kg marginally reduced % time in this zone [$t(13) = 1.68$, $P = 0.06$, one tailed], but there was no significant difference between 0.5 mg/kg ketamine versus saline, or between doses [maximum $t(9) = 1.13$, NS].

Fig. 1



Data are shown by drug treatment group (a–c: saline, 0.5 mg/kg ketamine, 3 mg/kg ketamine; d–f: saline, 0.05 mg/kg psilocin, 0.075 mg/kg psilocin). (a, d) Frequency of arm entries for maze zones (open and closed arms, center, and total); (b, e) mean percentage time spent in open and closed arms and the central square; and (c, f) mean distance traveled per visit to open and closed arms. Error bars show ± 1 SEM. Small asterisks indicate a significant (one tailed) difference from saline at $P<0.05$. Large asterisks indicate significant (two tailed) differences from saline at $P<0.05$. Small hash indicates marginally significant (one tailed) difference from saline. Connecting lines show significant differences between doses, one tailed, $P<0.05$.

There was a significant effect of drug treatment on the mean distance traveled per visit to the open arms [$F(2, 20)=4.00$, $P=0.04$; Fig. 1c]; *t*-tests showed that 0.5 mg/kg increased the distance traveled in open arms [$t(14)=3.26$, $P<0.01$], but 3 mg/kg did not (and there was no difference between doses) [$t(13)=1.11$, NS]. By contrast, drug treatment did not affect the mean distance traveled per visit to the closed arms [maximum $F(2, 20)=0.15$, NS], nor did it affect total distance traveled [$F(2, 20)=0.53$, NS (data not shown)].

Analysis of ethological data showed no effect of ketamine on total frequency of stretch-attend postures or head dips [maximum $F(2, 20)=1.34$, NS]. There was a marginal effect of ketamine on total frequency of rears [$F(2, 20)=2.18$, $P=0.07$, one tailed], manifested as increased rears at the low dose [$t(13)=1.87$, $P<0.05$, one tailed]. The high dose was not significantly different from saline, nor were there differences between doses [maximum $t(14)=1.41$, NS]. See Table 1 for descriptive statistics.

Table 1 Total mean frequencies (and SE in italics) of rears, head dips, and stretch-attend postures after saline, 0.5-mg/kg ketamine, 3-mg/kg ketamine, 0.05-mg/kg psilocin, and 0.075-mg/kg psilocin

	Ethological behaviors		
Drug treatments	Rears	Head dips	Stretch attend
Saline	21.13 (*2.81*)	11.25 (*2.53*)	17.00 (*1.80*)
0.5-mg/kg ketamine	28.00 (*2.24*)[a]	13.00 (*1.02*)	14.71 (*1.73*)
3-mg/kg ketamine	26.00 (*2.00*)	8.63 (*2.06*)	18.75 (*1.62*)
0.05-mg/kg psilocin	24.29 (*1.57*)	9.71 (*3.58*)	16.71 (*1.19*)
0.075-mg/kg psilocin	26.25 (*2.36*)	7.88 (*1.22*)	17.50 (*1.58*)

[a]A significant (one tailed) difference from saline.

Psilocin

Means suggested that psilocin reduced the number of entries into the open arms; however, the main effect was

marginal [$F(2, 21) = 2.02$, $P = 0.07$, one tailed; Fig. 1d]. As with ketamine, we followed up the marginal effect with t-tests, which showed that 0.05-mg/kg psilocin marginally reduced open arm entries [$t(14) = 1.87$, $P < 0.05$, one tailed], but 0.075 mg/kg did not, nor was the difference between doses significant [maximum $t(14) = 1.17$, NS]. Drug treatment did not significantly affect total arm entries, nor entries into the closed arms or central square [maximum $F(2, 21) = 0.68$, NS].

There was a significant effect of drug treatment on % time in open arms and closed arms [minimum $F(2, 21) = 2.60$, $P < 0.05$, one tailed; Fig. 1e]. Independent t-tests showed a reduction in % open arm time, and concomitant increase in % closed arm time that was significant for the 0.075 mg/kg psilocin dose [minimum $t(14) = 2.15$, $P < 0.03$, one tailed] and marginal for the 0.05 mg/kg dose [maximum $t(14) = 1.53$, $P = 0.07$, one tailed]. Psilocin affected % time in the central square [$F(2, 21) = 2.13$, $P = 0.07$, one tailed]. We followed up this marginal effect: t-tests showed that 0.075-mg/kg psilocin reduced % time in the central zone [$t(14) = 1.89$, $P < 0.04$, one tailed], but 0.05 mg/kg did not [$t(14) = 0.51$, NS]. There was also a significant difference between doses [$t(14) = 1.83$, $P < 0.04$, one tailed].

Drug treatment did not significantly affect total distance traveled in the maze [$F(2, 21) = 0.10$, $P = 0.90$ (data not shown)], nor mean distance traveled per visit to open or closed arms [maximum $F(2, 21) = 1.66$, NS; Fig. 1e]. Psilocin had no effect on total frequency of stretch-attend postures, or on rears [maximum $F(2, 20) = 1.25$, NS; Table 1].

Discussion

Our main finding was that subchronic intermittent microdosing with ketamine and psilocin (particularly at the lower doses) appeared to produce a mildly anxiogenic behavioral profile, as shown on the classic EPM criterion: reduced open arm entries. Although effects were modest, they are unlikely confounded by nonspecific motor effects (there was no concomitant increase in closed arm entries), by an atypical control group, nor by inadequate experimental parameters (saline-treated rats, as expected, spent ~30% of their arm time in open arms; File *et al.*, 2000).

Ketamine

Evidence that ketamine at 0.5 mg/kg resulted in anxiogenesis comes from the significant reduction in open arm entries (in the absence of a reduction in total or closed arm entries; Pellow *et al.*, 1985; Pellow and File, 1986; File, 1992). It is possible that, had the sample size been larger, the trend in reduced open arm time may also have reached significance (as it did for psilocin). However, 0.5 mg/kg of ketamine also increased the distance traveled per visit to the open arms, which does not immediately appear to be consistent with anxiogenesis, and although there was very little evidence that ketamine affected locomotor measures, there was a marginal increase in rears after this dose. It has been argued that effects on DA may underpin the antidepressant effects of (full doses of) ketamine (Can *et al.*, 2016); therefore, these findings (increased distance per open arm visit and increased rears) might also reflect antidepressant action (perhaps as increased motivation). It seems unlikely to reflect anxiolysis: first, because open arm distance is not an accepted measure of this, and second, this dose reduced open arm entries (which is the only generally agreed upon measure of anxiety). Moreover, there were no changes in head-dipping or stretch-attend posture frequencies that would indicate anxiolysis (or, by the same token, would strengthen the argument for anxiogenesis).

There was no clear effect of 3 mg/kg ketamine, with only a marginal reduction in percentage time in the center zone, which, although unconvincing in and of itself, does tend to suggest reduced exploration (if anything), which is consistent with anxiogenesis. Full doses of ketamine have produced inconsistent results in animal tests of anxiety (including the EPM), with some showing anxiogenesis (Babar *et al.*, 2001; Becker *et al.*, 2003), some anxiolysis (Silvestre *et al.*, 2002), and others null results (Becker *et al.*, 2003; Hayase *et al.*, 2006). Although methodological differences might account for such inconsistencies (Engin *et al.*, 2009), it may also be the case that some tests of anxiety are not suitable for detecting effects of ketamine reliably. Conditioned fear paradigms seem to produce more consistent effects; however, it is difficult to eliminate confounding effects such as learning (Engin *et al.*, 2009). Taken together, our findings indicate that ketamine is mildly anxiogenic on the EPM or that its effects are unclear in this test. We do not believe we have evidence of anxiolysis, despite an increase in distance traveled per visit to the open arms.

Psilocin

Psilocin at 0.05 mg/kg resulted in significantly reduced open arm entries (with no concomitant reduction in closed arm entries, which would indicate hypolocomotion), which suggests anxiogenesis. In line with this interpretation, rats also spent marginally less time in the open arms. However, this dose did not reduce head dips or stretch-attend postures, which would have further supported this conclusion. Psilocin at 0.075 mg/kg decreased time spent in open arms and in the central square and increased time spent in the closed arms. In addition, means suggested a trend in the direction of reduced open arm entries. Although it has been found previously that psilocin can reduce locomotion in the open field (Tylš *et al.*, 2016), this was at much higher doses. It is therefore unlikely that the effect of psilocin on distribution of time in the different maze zones is confounded by a locomotor effect (moreover, there was no effect on rears). Taken together, the findings suggest that 0.05 mg/kg psilocin produced mild anxiogenesis.

Data for 0.075 mg/kg were also consistent with anxiogenesis, although nonsignificant on the key measure of open arm entries. Neither dose showed any evidence of anxiolysis or locomotor effects. However, it should be noted that the vehicle control group for psilocin was not ideal, as the vehicle for drug contained a minute amount of acid, but the control vehicle did not.

Ketamine and psilocin

Vollenweider (2001) argued that ketamine and psilocin in humans act by a final common pathway, and each result in similar pharmacological effects. These are characterized by hyperactivation of the prefrontal cortices as well as striatal, temporo-parietal, and thalamic regions. At psychedelic doses, both serotonergic hallucinogens and dissociative anesthetics disrupt PPI in rodents by (direct or indirect) effects on cortico-striato-thalamic 5-HT_{2A} receptors (Vollenweider, 2001). 5-HT_{2A} receptors therefore might underlie the similarity of behavior effects that we observed here across psilocin and ketamine.

Behavioral effects for both compounds were observed drug free, 48 h after the final drug administration, when acute pharmacological effects of the drugs had subsided. In Wistar rats, serum and brain levels of ketamine after 30 mg/kg, intraperitoneally decrease to almost baseline within 2 h, with behavioral effects subsiding within 30 min (Páleníček *et al.* 2011); the elimination half-life (in Sprague-Dawley rats) for 125 mg/kg intraperitoneally (anesthetic dose administered with 10 mg/kg xylazine) is ~2 h (Veilleux-Lemieux *et al.* 2013). It would seem unlikely that biologically active metabolites of ketamine explain behavioral effects, as in rats (after 10 mg/kg, intraperitoneally), levels of hydroxynorketamines, norketamine, and dehydronorketamine in brain tissue and plasma had declined to zero by 480 min (Can *et al.*, 2016). Tizabi *et al.* (2012) reported clear neuroadaptive changes (e.g. increased hippocampal NMDA/AMPA receptor density) after 10 low, chronic, daily doses of 0.5 or 2.5 mg/kg ketamine (although only in female, but not male, Wistar-Kyoto rats), illustrating a possible mechanism by which ketamine might exert lasting effects on behavior.

The elimination half-life of psilocin in rats is 117.3 ± 40.3 min (Chen *et al.*, 2011); however, the time scale for the diminution of acute behavioral effects is not known. Nevertheless, Rambousek *et al.* (2014) found that some behavioral effects of psilocin that can persist for days beyond the acute pharmacological phase (albeit on conditioned rather than unconditioned avoidance behavior). In the case of psilocybin (in humans), psilocin was approaching the limit of detection after 24 h; however, psilocin-O-glucuronide remained theoretically higher at this time point; therefore, the possibility that behaviorally active metabolites might still be present after 48 h cannot be excluded (Hasler *et al.*, 2002). Alternatively, the psychedelic compound, 2,5-dimethoxy-4-iodoamphetamine, which is also an agonist for 5-HT receptors, including 5-HT_{2A}, has been shown to facilitate the rapid formation and reorganization of synapses (Jones *et al.*, 2009). Thus, although the precise mechanism(s) that might underlie our findings (or their apparent abiding nature) are as yet unknown, events involving 5-HT synapses (in particular, involvement of 5-HT_{2A} receptors), which in the case of ketamine is also likely to involve events involving (or interaction with) NMDA receptors, would seem likely.

Conclusion

Intermittent microdoses of ketamine or psilocin can affect anxiety; in humans, this effect seems to be relief from anxiety, but in a preclinical pharmacological screen, we observed mild anxiogenesis. Our statistical findings are modest, although the patterns of means for ketamine and psilocin were remarkably similar across all of the dependent variables that were measured, suggesting that we may have observed a small, but 'psychologically significant' effect. Nevertheless, we remain cautious in our conclusions; our findings are based on a single test, and it is known that drugs operating by 5-HT-ergic mechanisms can produce weak or variable effects in the EPM (Handley and McBlane, 1993). It would be useful to establish effects in other animal models of anxiety, as well as examine effects in models of depression.

Empirical work with ketamine and psilocybin/psilocin can be complicated by the obvious psychoactive effects in humans that damage 'double-blinding' in clinical trials, and confounded by nonspecific motor effects in animal studies (Engin *et al.*, 2009). Our intermittent microdosing paradigm limits psychomimetic and motor effects in preclinical studies (as very low doses are used) and further reduces their effects by screening for effects drug free. Further work should establish the most suitable preclinical screen for psychedelic microdoses, seek to confirm present findings, and determine any neuropsychological mechanism(s) responsible. Given the apparent 'trendiness' of self-medication by microdosing, it is especially crucial that more scientific knowledge is acquired to provide harm reduction and general guidance to those who choose to self-medicate, as well as to inform theory and clinical practice.

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Conflicts of interest

There are no conflicts of interest.

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