# RESEARCH ARTICLE

# Effects of psilocybin on hippocampal neurogenesis and extinction of trace fear conditioning

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**Abstract** Drugs that modulate serotonin (5-HT) synaptic concentrations impact neurogenesis and hippocampal (HPC)-dependent learning. The primary objective is to determine the extent to which psilocybin (PSOP) modulates neurogenesis and thereby affects acquisition and extinction of HPC-dependent trace fear conditioning. PSOP, the 5-HT<sub>2A</sub> agonist 25I-NBMeO and the 5-HT<sub>2A/C</sub> antagonist ketanserin were administered via an acute intraperitoneal injection to mice. Trace fear conditioning was measured as the amount of time spent immobile in the presence of the conditioned stimulus (CS, auditory tone), trace (silent interval) and post-trace interval over 10 trials. Extinction was determined by the number of trials required to resume mobility during CS, trace and post-trace when the shock was not delivered. Neurogenesis was determined by unbiased counts of cells in the dentate gyrus of the HPC birthdated with BrdU co-expressing a neuronal marker. Mice treated with a range of doses of PSOP acquired a robust conditioned fear response. Mice injected with low doses of PSOP extinguished cued fear conditioning significantly more rapidly than high-dose PSOP or saline-treated mice. Injection of PSOP, 25I-NBMeO or ketanserin resulted in significant dose-dependent decreases in number of newborn neurons in hippocampus. At the low doses of PSOP that enhanced extinction, neurogenesis was not decreased, but rather tended toward an increase. Extinction of "fear conditioning" may be mediated by actions of the drugs at sites other than hippocampus such as the amygdala, which is known to mediate the perception of fear. Another caveat is that PSOP is not purely selective for 5-HT<sub>2A</sub> receptors. PSOP facilitates extinction of the classically conditioned fear response, and this, and similar agents, should be explored as potential treatments for post-traumatic stress disorder and related conditions.

**Keywords** Neurogenesis · Psilocybin · Serotonin · Hippocampus · Learning · Memory · Trace conditioning

# Introduction

Psilocybin (4-phosphoryloxy-N,N-dimethyltryptamine, PSOP) was first isolated from Psilocybe mexicana, a mushroom from Central America by Albert Hofmann in 1957 and soon after produced synthetically in 1958 (Hofmann et al. 1958a, b). PSOP is an indole hallucinogen with potential clinical applications in the treatment for anxiety disorders, obsessive-compulsive disorder, major depression and cluster headaches (Moreno et al. 2006; Grob et al. 2011; Young 2013). PSOP is dephosphorylated in the body and converted into the active metabolite psilocin (4-hydroxy-N,N-dimethyltryptamine) which exerts psychoactive effects by altering neurotransmission through serotonin (5-HT) receptors 5-HT<sub>1A</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub>, but binds to 5-HT<sub>2A</sub> receptors with high affinity (Ki = 6 nM) (McKenna et al. 1990; Passie et al. 2002). 5-HT<sub>2A</sub> receptors are highly expressed throughout the hippocampus (HPC) in

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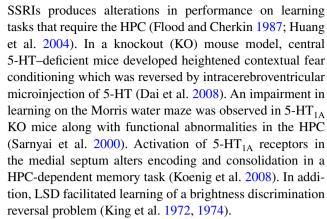
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the dentate gyrus (DG), hilus, cornu ammonis (CA) 1 and CA3 and are colocalized on GABAergic neurons, pyramidal and granular cells (Luttgen et al. 2004; Morilak et al. 1993, 1994; Pompeiano et al. 1994; Shen and Andrade 1998; Cornea-Hebert et al. 1999). 5-HT<sub>2A</sub> receptor downregulation is thought to be an adaptive process triggered by chronic administration of selective 5-HT uptake inhibitors (SSRIs) (Eison and Mullins 1996).

Antidepressant medications which elevate 5-HT over prolonged periods such as SSRIs have been shown to enhance hippocampal neurogenesis (Malberg et al. 2000). Neurogenesis occurs throughout the life span in the adult brain, the subventricular zone (SVZ) and the subgranular zone (SGZ) in the DG (Altman 1962, 1969; Altman and Das 1965). The proliferation and survival of neural progenitors in the adult HPC can be influenced by a variety of stimuli including stress, age, drugs of abuse, physical activity and depression (Malberg et al. 2000; Van et al. 1999; Kempermann et al. 1998; Gould et al. 1992). The involvement of 5-HT in the regulation of neurogenesis may be mediated through different 5-HT receptor subtypes expressed on cells in the neurogenic microniche (Barnes and Sharp 1999).

Serotonin activates fifteen known receptors, many of which are expressed in the DG (Tecott et al. 1993; Vilaro et al. 1996; Djavadian et al. 1999; Clemett et al. 2000; Kinsey et al. 2001). Acute activation of the 5-HT<sub>1</sub> receptor has been shown to increase cell proliferation in the DG, whereas repeated inhibition of the receptor results in diminished neurogenesis (Klempin et al. 2010). Interestingly, both acute and repeated stimulation of the 5-HT<sub>2</sub> receptor attenuate proliferation and neuronal survival (Klempin et al. 2010). Studies investigating the effects of 5-HT<sub>2</sub> receptor subunits on proliferation and neurogenesis in the DG have found that acute activation of the 5-HT<sub>2A/C</sub> receptor via 2,5-dimethoxy-4-iodoamphetamine (DOI), the 5-HT<sub>2C</sub> receptor agonist RO 600175 or the 5-HT<sub>2C</sub> receptor antagonist SB-206553 had no effect on cell proliferation, whereas the 5-HT<sub>2A/C</sub> receptor antagonist ketanserin produced a 63 % decrease in BrdU incorporation (Banasr et al. 2004). Similarly, other investigators reported that acute ketanserin decreased proliferation, but chronic ketanserin increased proliferation in the DG (Jha et al. 2008). Additionally, no effect on proliferation was observed after DOI or lysergic acid diethylamide (LSD) was administered either acutely or once daily for seven consecutive days (chronic) (Jha et al. 2008). Administering LSD and PSOP by giving daily doses has been shown to produce rapid tolerance to the drug and results in a selective downregulation of the 5-HT<sub>2A</sub> receptor (Buckholtz et al. 1985, 1990).

The serotonergic system has been implicated in hippocampal (HPC)-dependent learning. Administration of



Evidence suggests that performance on HPC-dependent learning tasks is influenced by neurogenesis in the DG of the HPC (Van et al. 2002; Gould et al. 1999a, b; Nilsson et al. 1999; Shors et al. 2001, 2002). This was elegantly demonstrated by Shors et al. by treating animals with methylazoxymethanol acetate (MAM), an antimitotic agent which eradicates the progenitor cell population in the DG before testing mice on HPC-dependent and HPCindependent learning tasks (Shors et al. 2001, 2002). MAM-treated animals had significantly fewer BrdU+ cells in the SGZ but showed no impairment in the spatial navigation task (HPC-dependent) or delay eyeblink conditioning task (HPC-independent), demonstrating that the hippocampal progenitor cell population is not essential for these particular tasks (Shors et al. 2001, 2002). In contrast, MAM severely impaired performance on trace fear conditioning and trace eyeblink conditioning, providing evidence for the involvement of progenitor cells in the DG in trace classical conditioning.

The present study investigated the role of PSOP through the 5-HT<sub>2A</sub> receptor on hippocampal neurogenesis and a HPC-dependent learning task, trace fear conditioning. The effects of single-dose injections of PSOP, the 5-HT<sub>2A</sub> agonist 25I-NBMeO and the 5-HT<sub>2A/C</sub> antagonist ketanserin on the survival and phenotypic fate of progenitor cells in the DG were assessed using immunofluorescence techniques. The effects of single doses of PSOP on trace fear conditioning were chosen for this study because it has previously been demonstrated to be a HPC-dependent learning paradigm.

#### Materials and methods

Animals

C57BL/6J male mice (30–40 g) were housed in standard laboratory cages and left undisturbed for 1 week after arrival at the animal facility. All mice had ad libitum access to water and laboratory chow and were maintained in a



temperature- and humidity-controlled room on a 12:12 light/dark cycle with light onset at 7:00 a.m. This study was carried out in strict accordance with the recommendations from the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee at the University of South Florida (IACUC #R3347).

# Drugs

25I-NBMeO (4-iodo-2,5-dimethoxy-*N*-(2-methoxybenzyl) phenethylamine) was synthesized in the laboratory of Dr. David Nichols (Braden et al. 2006). PSOP was provided by Dr. Francisco Moreno from the University of Arizona. Ketanserin (+)-tartrate salt (#S006, St. Louis, MO) and 5-bromo-2'-deoxyuridine (#B5002, St. Louis, MO) were supplied by Sigma-Aldrich Inc.

# General procedure

A total of 48 C57BL/6 mice received a single injection of 0.1 mg/kg PSOP (n=6), 0.5 mg/kg PSOP (n=6), 1.0 mg/kg PSOP (n=6), 0.1 mg/kg 25I-NBMeO (n=6), 0.3 mg/kg 25I-NBMeO (n=6), 0.3 mg/kg 25I-NBMeO (n=6), 1.0 mg/kg 25I-NBMeO (n=6), 1.0 mg/kg ketanserin (n=6) or saline (n=6). Mice received an intraperitoneal (i.p.) injection of 75 mg/kg BrdU once daily for 4 days following drug administration and were euthanized 2 weeks after drug injection. Mice were euthanized with nembutal and then transcardially perfused with 0.9 % saline followed by 4 % paraformaldehyde. Brains were stored in 4 % paraformaldehyde, transferred to 20 % sucrose solution, sectioned coronally using a cryostat (Leica, Germany) at 30  $\mu$ M in a 1:6 series and stored in 24-well plates in cryoprotectant at -20 °C.

# Trace fear conditioning

Mice (n = 9-10/condition) received an i.p. injection of PSOP (0.1, 0.5, 1.0 and 1.5 mg/kg), ketanserin (1.0 mg/ kg) or 0.9 % saline and 24 h later were habituated to the testing chamber for 30 min. The fear conditioning environment consisted of two chambers each placed inside a larger soundproof chamber. The 35.6 (W) × 38.1 (D) × 31.8 (H) cm freeze monitor box (San Diego Instruments, San Diego, CA) is a clear Plexiglas chamber with a removable lid which contains a metal grid floor (0.3-cm grids spaced 0.8 cm apart) through which a foot shock can be delivered. Photo-beam activity within the chamber recorded the vertical and horizontal movements of mice. Two minutes into the habituation period, a baseline (BL) measure of movement was recorded for 3 min and served as the habituation BL measure. Freeze monitor boxes were cleaned with quatricide between each mouse to prevent olfactory cues. Mice were returned to their home cage after habituation.

The next day mice were returned to the same freeze monitor chamber and underwent training to form CS–US associations. After a 2-min acclimation period, mice were exposed to 10 trials of trace fear conditioning, which is illustrated in Fig. 2. Each trial consisted of the CS (tone, 82 dB, 15 s) followed by a trace interval (30 s) and ended with the presentation of the US (shock, 0.5 s, 1 mA) delivered through the grid flooring. After each trial ended, there was a 210-s intertrial interval (ITI). Freeze monitor boxes were cleaned with quatricide between each mouse to prevent olfactory cues, and mice were returned to their home cage after training.

On day 3 of the task, retention and extinction of the conditioned fear response were assessed in three phases. First, mice were placed in the freeze monitor box for 5 min, and movement was recorded for the last 3 min and used to assess the measure of fear (immobility) associated with the training context. Mice were then returned to the home cage for 1 h. Second, the context was altered by replacing the grid floors with black Plexiglas flooring and adding a cotton ball with 1 ml vanilla essence inside the sound-attenuated chamber. Mice were placed inside the novel chamber, and after 2 min, movements were recorded for the next 3 min.

In the third phase, extinction of the conditioned fear response was determined by placing the animals in the freeze monitor boxes and running ten trials of CS presentation (auditory tone for 15 s) followed by a trace of silence for 30 s without delivery of the UCS (shock). As with the acquisition phase of the learning, a 210-s ITI separated each trial. The conditioned fear response was expressed as the percentage of time the mice were immobile during CS (tone; 15 s), during the trace interval (30 s) and for 30 s after the trace interval.

# Immunofluorescence

For the double labeling of progenitor cells in the DG, free-floating sections were denatured using 2N HCl and neutralized in 0.15 M borate buffer and then washed in PBS. Tissue was blocked in PBS+ (PBS, 10 % normal goat serum, 1 % 100× Triton X, 10 % BSA) for 1 h at 4 °C and incubated for 48 h at 4 °C in an antibody cocktail of rat monoclonal anti-BrdU (AbD Serotec, Raleigh NC, #OBT0030G, 1:100) plus mouse anti-NeuN (Chemicon, 1:100) in PBS plus 2 % normal goat serum. Sections were washed in PBS and incubated in a secondary antibody cocktail of goat anti-rat IgG Alexa Fluor 594 (1:1,000, Invitrogen, Eugene OR) plus goat antimouse IgG Alexa Fluor 488 (1:400, Invitrogen) and coated with Vectashield mounting medium with DAPI (Invitrogen).



#### Quantitation

For the quantification of double-labeled cells using immunofluorescence, the number of BrdU+- and BrdU/NeuN+-labeled cells was estimated using every sixth section taken throughout the DG (every 180 microns). To avoid counting partial cells, a modification to the optical dissector method was used so that cells on the upper and lower planes were not counted. The number of BrdU+ cells counted in every sixth section was multiplied by six to get the total number of BrdU+ or BrdU/NeuN+ cells in the DG (Shors et al. 2002). Positive labeling was verified by confocal microscopy (Zeiss Model LSM510).

# Design and statistical analyses

One-way analysis of variance (ANOVA) was used to evaluate the effects of PSOP, the 5-HT<sub>2A</sub> receptor agonist and an antagonist on hippocampal neurogenesis (proliferation and survival). Separate two-way repeated-measures ANOVA was used to evaluate the effect of Dose and Trial on each dependent variable in the trace fear conditioning task. Dependent measures recorded included percentage freezing during CS, during trace, after trace, during habituation baseline, during the context text and in response to the novel environment. When appropriate, post hoc analyses such as Bonferroni were used to isolate effects. All statistical analyses were determined significantly at the 0.05 alpha level.

# Results

Effects of PSOP administration on trace fear conditioning

# Acquisition of the conditioned fear response

Mice were placed in the freeze monitor box 24 h after injection of a single dose of PSOP (0.1, 1.0 and 1.5 mg/kg), ketanserin (1.0 mg/kg) or saline vehicle. Training consisted of a sequence of 10 trials of CS–UCS presentation. A single trial consisted of a 15-s auditory cue (CS) followed by a silent "trace" for 30 s terminating in a brief shock to the feet. The intertrial interval (ITI) was 210 s. After just a few trials, animals acquired the conditioned response, that is, freezing during presentation of the CS, trace and post-shock interval (Fig. 1). In trial 3, mice treated with low-dose PSOP (0.1 mg/kg) exhibited a trend toward less freezing (Fig. 1d). Two-way ANOVA showed a significant effect of Trial [F(2,102) = 7.83, p < 0.0007] with trials 2 and 3, eliciting significantly more freezing in response to the CS compared to trial one, but no significant effect of

dose. Post hoc comparisons of each dose to vehicle, with Bonferroni correction, also did not reveal a significant difference between PSOP doses and saline. By trial 10, all mice froze for the same amount regardless of dose or drug treatment (Fig. 1e).

# Contextual fear conditioning

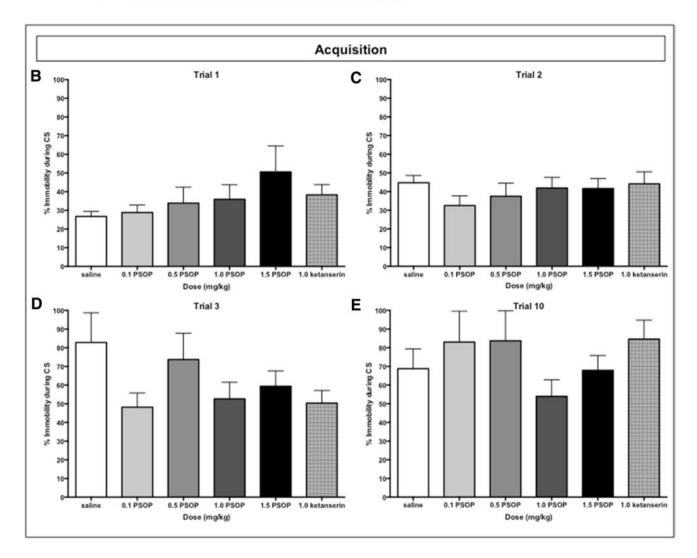
Contextual fear conditioning was assessed by comparing percentage immobility in the freeze monitor box on habituation day to percentage immobility during the context test. There was no interaction between Dose and Trial  $[F(5,90)=0.95,\ p=0.45]$  and no effect of Dose during the habituation BL or context test  $[F(5,90)=1.15,\ p=0.34]$ . Notably, there was a significant effect of Trial  $[F(1,90)=105.85,\ p<0.0001]$ , indicating that mice spent significantly more time freezing after the CS–US pairings during the context test compared to habituation baseline. These data show that all mice learned to fear the context in which they received the CS–US pairings (Fig. 2a, b).

# Extinction of the conditioned fear response

A measure of the strength or durability of the conditioned fear response is the number of trials required to extinguish the conditioned response. This was determined by running a set of 10 extinction trials during which the CS and trace were not followed by shock. Two-way repeatedmeasures ANOVA of freezing after the trace interval revealed a significant interaction between Dose and Trial [F(20,216) = 2.68, p < 0.0002]. Mice that received low doses of PSOP (0.1 and 0.5 mg/kg) responded robustly to the CS and trace interval as well as to immediate posttrace interval of 30 s by freezing significantly greater than mice treated with vehicle in the very first extinction trial, indicating they "remembered" the link between the CS and the shock (Figs. 2c, 3c). However, by trial 3, the mice treated with low-dose PSOP no longer froze in the absence of the UCS, but adapted quickly to the new condition in which the CS and trace did not culminate in shock (Fig. 2e). Such rapid extinction of the fear response was not observed in mice treated with higher PSOP doses or with ketanserin. Mice that received higher doses of PSOP eventually decreased freezing, so that by trial 10 they no longer exhibited a fear response (Fig. 2f). Ketanserin treatment also produced a robust fear response through trial 3, but as with all mice treated with either PSOP or ketanserin, the fear response was significantly decreased compared to control by trial 10. These results indicate that low-dose PSOP facilitates extinction of the fear response in a hippocampal-dependent trace conditioning paradigm.







**Fig. 1** Effects of psilocybin on acquisition of trace fear conditioning. **a** Schematic representation of the trace fear conditioning paradigm. Trace fear conditioning is a HPC-dependent task in which the presentation of the conditioned stimulus (CS, tone) is separated in time by a trace interval to the unconditioned stimulus (US, shock). **b–e** Percentage immobility during the CS is expressed on the Y axis and dose on the X axis. After trial 2 (**c**) animals acquired the conditioned response, freezing 34–45 % of the time during presentation of the CS.

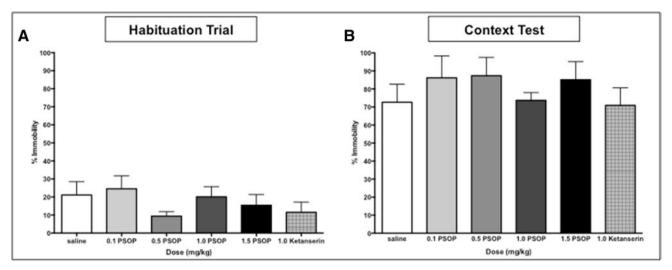
A quantitatively similar freezing response was observed during the trace and 30 s after the trace (data not shown). By trial 10 (**d**) all mice froze 55–80 % of the CS time interval regardless of drug dose. Two-way ANOVA showed a significant effect of Trial [F(2,102) = 7.83, p < 0.0007] with trials 2 and 3 eliciting significantly more freezing in response to the CS compared to trial 1 and dose [F(5,51) = 4.96, p < 0.0009]

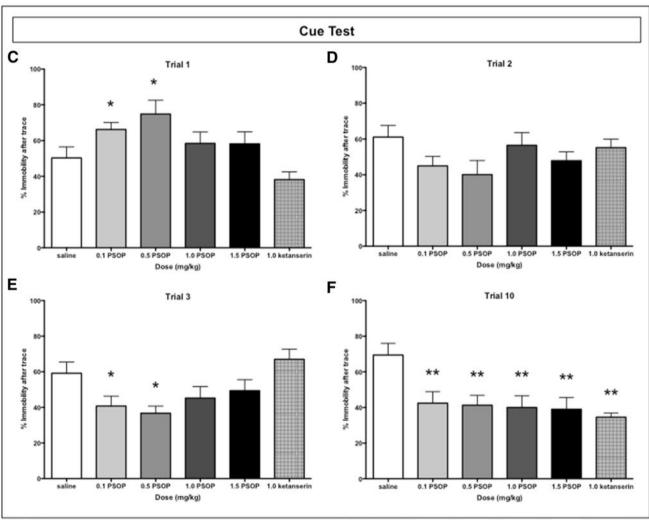
Effects of PSOP, 25I-NBMeO and ketanserin in vivo on cell survival and neurogenesis in the hippocampus

To investigate the effects of a single dose of PSOP, the 5-HT2a receptor agonist 25I-NBMeO or antagonist ketanserin on cell survival and neurogenesis, mice (n = 6-7 per condition) were injected with PSOP (0.1, 0.5 or 1.0 mg/kg), 25I-NBMeO (0.1, 0.3 or 1.0 mg/kg), ketanserin (1.0 mg/

kg) or 0.9 % saline solution followed by injections of the cellular birth-date marker BrdU (75 mg/kg) once daily for 4 days after drug administration. Animals were euthanized 2 weeks after drug injection. PSOP treatment produced a dose-dependent decrease in the number of surviving BrdU+ cells (Fig. 3). ANOVA showed a significant effect of dose on birth-dated cell survival, and post hoc comparison between doses and vehicle revealed a statistically







significant decrease in cell survival following high doses of PSOP but not following the low doses of 0.1 and 0.5 mg/kg (Fig. 3a). The phenotypic fate of BrdU-birth-dated cells was determined by immunofluorescent double labeling of

BrdU+ and NeuN+ cells. There was a biphasic response of PSOP on neurogenesis, with the lowest dose (0.1 mg/kg) increasing number of BrdU/NeuN+ cells and the high dose (1 mg/kg) decreasing number of new neurons (Fig. 3b).



◆Fig. 2 Contextual fear conditioning and extinction. a Percentage immobility expressed during exposure to the freeze monitor box during habituation and b during the context test. All mice expressed contextual fear conditioning as indicated by a significant increase in percentage immobility during the context test (p < 0.05). **c-f** Cue test (presentation of CS and trace without shock; extinction protocol). Y axis shows percentage of time mice were immobile during the 30-s interval after the shock would have been delivered. Two-way repeated-measures ANOVA of freezing after the trace interval revealed a significant interaction between Dose and Trial [F(20,216) = 2.68, p < 0.0002]. c Mice treated with low-dose PSOP exhibited significant freezing on trial 1 of the extinction protocol. d, e By trials 2 and 3, low-dose-treated mice exhibited significantly less immobility. Control mice demonstrated robust fear conditioning throughout the entire test, whereas mice treated with PSOP or ketanserin froze significantly less by trial 10, indicating rapid extinction of the fear response. \*Indicates a significant difference from ketanserin, \*\*indicates a significant difference from saline

One-way ANOVA revealed a highly significant effect of PSOP dose on the number of double-labeled neurons in the DG. Despite the trend toward stimulation of neurogenesis by 0.1 mg/kg PSOP, post hoc comparison of PSOP doses to vehicle did not reach statistical significance, but the depression of neurogenesis by the high dose of PSOP reached statistical significance. Ketanserin, like high-dose PSOP, also significantly decreased neurogenesis (number of BrdU/NeuN+ cells) compared to saline.

Injections of 25I-NBMeO (0.1, 0.3 and 1 mg/kg) or of the antagonist ketanserin resulted in a significant decrease in the number of surviving BrdU+ cells and BrdU/NeuN+ neurons in hippocampus compared to saline injections (Fig. 3c, d). Unlike the PSOP injections, low doses of 25I-NBMeO did not produce a trend toward increased neurogenesis.

# Discussion

The present investigation demonstrates that PSOP produces alterations in hippocampal neurogenesis and in a HPC-dependent learning paradigm of cue-associated fear conditioning. To evaluate the effects of PSOP on HPC-dependent learning, an acute treatment of PSOP or ketanserin was administered prior to trace fear conditioning. During the acquisition of the conditioned response, there was a striking increase in the amount of time spent freezing during the presentation of the CS and during the trace period and immediate post-trace interval from trial 1 to trial 3. These data demonstrate that the association between the CS and US promoted freezing in anticipation of the shock as well as during the period after the shock. Notably, the acquisition of the conditioned response was not impaired by acute administration of PSOP or ketanserin.

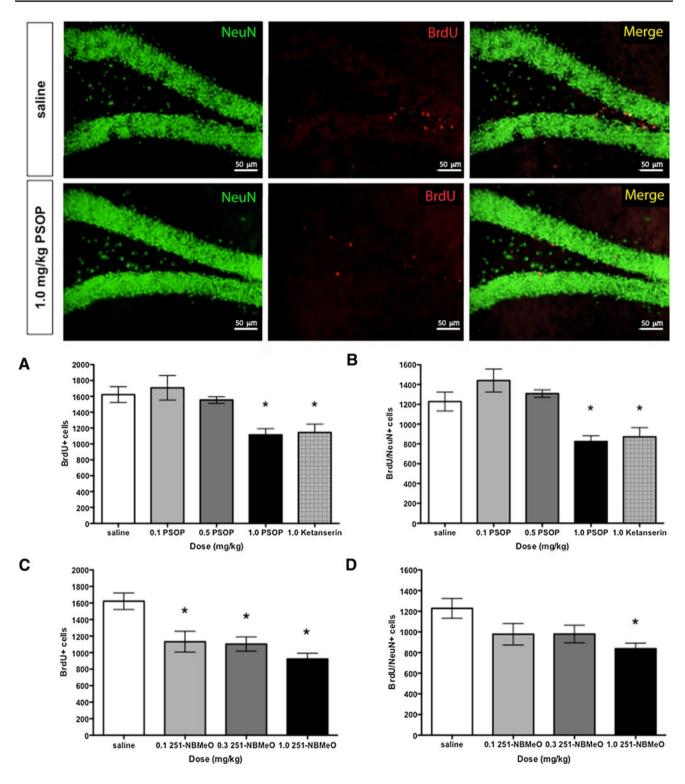
Regardless of the PSOP dose, mice displayed similar locomotor activity levels in the freeze monitor box during

the habituation baseline exposure and during re-exposure to the same environment during the contextual fear conditioning test. As expected, mice froze substantially more during re-exposure to the same context after CS-US associations were formed compared to the habituation baseline, indicating that mice formed contextual fear conditioning regardless of their drug exposure. It is well known that contextual fear conditioning is a HPC-dependent learning task (Hirsh 1974; Kim and Fanselow 1992; McNish et al. 1997; Frohardt et al. 1999; Esclassan et al. 2009). The serotonergic system has been implicated in performance on the contextual fear conditioning task (Dai et al. 2008), and hippocampal neurogenesis is required for the expression of fear memory (Shors et al. 2001, 2002). The present investigation found that interaction with the 5-HT<sub>2A</sub> receptor does not impair contextual fear conditioning since no differences were observed between controls and mice treated with PSOP or ketanserin.

Unlike the acquisition of the conditioned fear response, where drug had no significant effect on learning the association between CS, trace and shock (UCS), the extinction of the conditioned response was accelerated in mice treated with low doses of PSOP. This rapid reversal of fear conditioning was only observed with low-dose PSOP treatment and was not present with higher doses or with ketanserin. Thus, PSOP treatment does not alter acquisition of a conditioned fear response nor in remembering the link between the CS and UCs (contextual test), but low-dose PSOP facilitates extinction by disassociating the link between the CS, trace and the UCS (shock). Given that low doses of PSOP accelerated fear extinction, it is interesting to consider whether PSOP is blunting the emotional component of the fear memory which leads to rapid extinction. We observed that all mice freeze in response to each presentation of the shock during training and assume that for all mice regardless of the treatment, fear is evoked. In human studies, low doses of PSOP produce no differences in general well-being, emotional excitability, anxiety-depressiveness, plasma cortisol levels, heart rate, body temperature and blood pressure (Hasler et al. 2004). Taken together, we believe that the PSOP facilitation of fear extinction is not likely due to blunted emotion but instead caused by diminished neurogenesis and alterations in hippocampal neurotransmission.

The molecular basis for creating the links between CS and UCS in the trace conditioning paradigm is not completely understood. Clearly, synaptic plasticity in the HPC is critical for the acquisition of new associations (learning) and recall of those associations (memory). Brain-derived neurotrophic factor (BDNF) has been implicated in synaptic plasticity and memory processing (Kang et al. 1997; Pang et al. 2004; Tyler et al. 2002) through the modulation of synapse formation and dendritic spine growth in





**Fig. 3** Effect of acute 5-HT<sub>2A</sub> receptor stimulation on hippocampal neurogenesis. Representative photomicrographs of NeuN+ cells (left), BrdU+ cells (*center*) and NeuN/BrdU+ cells (*right*) in the dentate gyrus from saline (*top*)- and 1.0 mg/kg PSOP (*bottom*)-treated mice, *scale* 50  $\mu$ M. Mice (n=6 per condition) were injected with **a, b** saline, PSOP (0.1, 0.5 or 1.0 mg/kg) or 1.0 mg/kg ketanserin **c, d** saline or 25I-NBMeO (0.1, 0.3 or 1.0 mg/kg) and then received 75 mg/kg BrdU once daily for 4 days after drug administration, followed by euthanasia 2 weeks after drug injection. **a, c** The

total number of BrdU+ cells in the DG was significantly diminished after a single injection of 1.0 mg/kg PSOP, 25I-NBMeO or ketanserin (p < 0.05). **b, d** Acute administration of 1.0 mg/kg PSOP, 1.0 mg/kg of 25I-NBMeO and ketanserin significantly diminished the number of BrdU/NeuN+ cells compared to saline (p < 0.05). These data suggest acute administration of 5-HT<sub>2A</sub> receptor decreases progenitor cell survival (and/or proliferation) and attenuates the generation of new neurons in the HPC. \*Indicates a significant difference from saline



the HPC (Bamji et al. 2006; Tyler and Pozzo-Miller 2001, 2003). Chronic administration of 5-HT agonists (including SSRIs) upregulates BDNF mRNA expression in the HPC (Nibuya et al. 1995, 1996). Evidence suggests that the 5-HT<sub>2A</sub> receptor is involved in the regulation of BDNF in the HPC (Vaidya et al. 1997). Specifically, DOI, a 5-HT<sub>2A/C</sub> receptor agonist, decreased BDNF mRNA expression in the granule cell layer of the DG, but not in the CA subfields of the HPC. Interestingly, the decrease in BDNF mRNA expression was blocked by the 5-HT<sub>2A</sub> receptor antagonist, but not the 5-HT<sub>2C</sub> receptor antagonist, implicating the 5-HT<sub>2A</sub> receptor in the regulation of BDNF expression (Vaidya et al. 1997). The process of forgetting or breaking synaptic links in HPC is even less understood. One possible explanation for the findings presented here is that single low doses of PSOP acting via the 5-HT<sub>2A</sub> receptors on HPC neurons inhibit BDNF expression and thereby decrease synaptic growth and neurogenesis.

The data reported demonstrate that the administration of PSOP produced a biphasic response in hippocampal neurogenesis; a low dose (0.1 mg/kg) resulted in a trend toward increased neurogenesis, whereas the high dose of PSOP significantly decreased the formation of new neurons measured 2 weeks after drug exposure. In addition, all doses of the potent 5-HT<sub>2A</sub> receptor agonist 25I-NBMeO and the 5-HT<sub>2A/C</sub> receptor antagonist ketanserin decreased hippocampal neurogenesis. A range of doses of ketanserin (1–5 mg/kg) administered within 4 h of killing has been reported to decrease the number of BrdU+ cells in the DG, indicating a reduction in cell proliferation (Banasr et al. 2004; Jha et al. 2008). The present study extends these findings by demonstrating that single doses of ketanserin decreased the number of BrdU+ and BrdU/NeuN+ cells 2 weeks after drug administration, indicating a reduction in both progenitor cell survival and formation of new neurons after exposure to a single dose of the 5-HT<sub>2A/C</sub> antagonist.

Using positron emission tomography (PET), Vollenweider et al. established that PSOP-induced schizophrenia, like psychosis, in humans is completely blocked by the 5-HT2a antagonist ketanserin, demonstrating that PSOP produces psychotropic effects mainly via 5-HT2a receptor activation (Vollenweider et al. 1998). However, PSOP administration decreases [11C]raclopride receptor binding potential in the basal ganglia, which is indicative of increased DA levels (Vollenweider et al. 1999). Additionally, the D2 antagonist haloperidol attenuates the psychotomimetic effects of PSOP, clearly demonstrating that the DA system is involved in PSOP-induced psychotomimesis (Vollenweider et al. 1999). Interestingly, increases in DA disrupt fear extinction (Willick and Kokkinidis 1995; Borowski and Kokkinidis 1998); therefore, it is plausible that DA may contribute to PSOP facilitating the extinction of the fear response. Morrow and colleagues demonstrated that DAergic neurons which project to the medial prefrontal cortex (PFC) are critical in fear extinction and reduced rates of extinction are observed after 6-hydroxydopamine lesions to the PFC reduced DA levels to 13 % of control (Morrow et al. 1999). Furthermore, the activation of 5-HT<sub>2A</sub> receptors in the medial PFC increases extracellular glutamate levels (Aghajanian and Marek 1999), thereby affecting subcortical neurotransmission and increasing the activity of DA neurons in the ventral tegmental area (VTA), resulting in increased DA transmission in mesocortical and mesostriatal regions (Vazquez-Borsetti et al. 2009). Since drugs that increase DA bioavailability are involved in fear extinction, it is plausible that PSOP is facilitating fear extinction through an interaction between 5-HT and DA neurotransmitter systems in PFC, amygdala and HPC.

To summarize, the data reported in the present investigation demonstrate that PSOP at low doses facilitates extinction of a HPC-dependent classically conditioned fear response. Low dose of PSOP was also associated with a trend toward an increase, or no change, in hippocampal neurogenesis compared to vehicle treatment. In contrast, higher doses of PSOP (or of the 5-HT<sub>2A/C</sub> antagonist ketanserin) significantly depressed neurogenesis, and these doses had no impact on extinction of the conditioned fear response. Although previous studies have reported that ablation of hippocampal neurogenesis with a potent chemotherapeutic agent impaired acquisition of HPC-dependent fear conditioning (Shors et al. 2001, 2002), the present study did not reveal impaired acquisition of the trace fear conditioning response, likely due to modest inhibition of neurogenesis with the range of PSOP doses used. Importantly, studies of the effects of ablation of hippocampal neurogenesis focused on acquisition of the response and did not address extinction of a previously learned conditioned fear response as was done here. It is possible that the effect of low-dose PSOP on hippocampal neurogenesis, which was minimal, may not be the critical mechanism for forgetting or erasing the link between the noxious stimulus and the conditioned stimulus. PSOP clearly interacts with 5-HT<sub>2A</sub> receptors in other regions of brain known to mediate the emotion of fear such as amygdala (Ledoux 2003). However, one cannot invoke reduction in the fear emotion by PSOP because the acquisition of the conditioned fear response was not affected by PSOP administration at any dose. A final caveat is that PSOP is not a pure 5-HT<sub>2A</sub> agonist and may interact with other 5-HT receptor subtypes. In conclusion, low-dose PSOP which acts primarily on the 5-HT<sub>2A</sub> receptor facilitates extinction of a conditioned fear response and raises the possibility of targeting the 5-HT<sub>2A</sub> receptor to treat conditions where a previously neutral set of stimuli are associated with noxious, or life-threatening events, such as post-traumatic stress disorder.



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# References

- Aghajanian GK, Marek GJ (1999) Serotonin, via 5-HT2A receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. Brain Res 825:161–171
- Altman J (1962) Are new neurons formed in the brains of adult mammals? Science 135:1127–1128
- Altman J (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137:433–457
- Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124:319–335
- Bamji SX, Rico B, Kimes N, Reichardt LF (2006) BDNF mobilizes synaptic vesicles and enhances synapse formation by disrupting cadherin-beta-catenin interactions. J Cell Biol 174:289–299
- Banasr M, Hery M, Printemps R, Daszuta A (2004) Serotonininduced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. Neuropsychopharmacology 29:450–460
- Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. Neuropharmacology 38:1083–1152
- Borowski TB, Kokkinidis L (1998) The effects of cocaine, amphetamine, and the dopamine D1 receptor agonist SKF 38393 on fear extinction as measured with potentiated startle: implications for psychomotor stimulant psychosis. Behav Neurosci 112:952–965
- Buckholtz NS, Freedman DX, Middaugh LD (1985) Daily LSD administration selectively decreases serotonin2 receptor binding in rat brain. Eur J Pharmacol 109:421–425
- Buckholtz NS, Zhou DF, Freedman DX, Potter WZ (1990) Lysergic acid diethylamide (LSD) administration selectively downregulates serotonin2 receptors in rat brain. Neuropsychopharmacology 3:137–148
- Clemett DA, Punhani T, Duxon MS, Blackburn TP, Fone KC (2000) Immunohistochemical localisation of the 5-HT2C receptor protein in the rat CNS. Neuropharmacology 39:123–132
- Cornea-Hebert V, Riad M, Wu C, Singh SK, Descarries L (1999) Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. J Comp Neurol 409:187–209
- Dai JX, Han HL, Tian M, Cao J, Xiu JB, Song NN, Huang Y, Xu TL, Ding YQ, Xu L (2008) Enhanced contextual fear memory in central serotonin-deficient mice. Proc Natl Acad Sci USA 105:11981–11986
- Djavadian RL, Wielkopolska E, Bialoskorska K, Turlejski K (1999) Localization of the 5-HT1A receptors in the brain of opossum *Monodelphis domestica*. NeuroReport 10:3195–3200
- Eison AS, Mullins UL (1996) Regulation of central 5-HT2A receptors: a review of in vivo studies. Behav Brain Res 73:177–181
- Esclassan F, Coutureau E, Di SG, Marchand AR (2009) Differential contribution of dorsal and ventral hippocampus to trace and delay fear conditioning. Hippocampus 19:33–44
- Flood JF, Cherkin A (1987) Fluoxetine enhances memory processing in mice. Psychopharmacology 93:36–43

- Frohardt RJ, Guarraci FA, Young SL (1999) Intrahippocampal infusions of a metabotropic glutamate receptor antagonist block the memory of context-specific but not tone-specific conditioned fear. Behav Neurosci 113:222–227
- Gould E, Cameron HA, Daniels DC, Woolley CS, McEwen BS (1992) Adrenal hormones suppress cell division in the adult rat dentate gyrus. J Neurosci 12:3642–3650
- Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ (1999a) Learning enhances adult neurogenesis in the hippocampal formation. Nat Neurosci 2:260–265
- Gould E, Tanapat P, Hastings NB, Shors TJ (1999b) Neurogenesis in adulthood: a possible role in learning. Trends Cogn Sci 3:186–192
- Grob CS, Danforth AL, Chopra GS, Hagerty M, McKay CR, Halber-stadt AL, Greer GR (2011) Pilot study of psilocybin treatment for anxiety in patients with advanced-stage cancer. Arch Gen Psychiatry 68:71–78
- Hasler F, Grimberg U, Benz MA, Huber T, Vollenweider FX (2004) Acute psychological and physiological effects of psilocybin in healthy humans: a double-blind, placebo-controlled dose-effect study. Psychopharmacology 172:145–156
- Hirsh R (1974) The hippocampus and contextual retrieval of information from memory: a theory. Behav Biol 12:421–444
- Hofmann A, Frey A, Ott H, Petr ZT, Troxler F (1958a) Elucidation of the structure and the synthesis of psilocybin. Experientia 14:397–399
- Hofmann A, Heim R, Brack A, Kobel H (1958b) Psilocybin, a psychotropic substance from the Mexican mushroom *Psilicybe mexi*cana Heim. Experientia 14:107–109
- Huang SC, Tsai SJ, Chang JC (2004) Fluoxetine-induced memory impairment in four family members. Int J Psychiatry Med 34:197–200
- Jha S, Rajendran R, Fernandes KA, Vaidya VA (2008) 5-HT2A/2C receptor blockade regulates progenitor cell proliferation in the adult rat hippocampus. Neurosci Lett 441:210–214
- Kang H, Welcher AA, Shelton D, Schuman EM (1997) Neurotrophins and time: different roles for TrkB signaling in hippocampal longterm potentiation. Neuron 19:653–664
- Kempermann G, Kuhn HG, Gage FH (1998) Experience-induced neurogenesis in the senescent dentate gyrus. J Neurosci 18:3206–3212
- Kim JJ, Fanselow MS (1992) Modality-specific retrograde amnesia of fear. Science 256:675–677
- King AR, Martin IL, Seymour KA (1972) Reversal learning facilitated by a single injection of lysergic acid diethylamide (LSD 25) in the rat. Br J Pharmacol 45:161P–162P
- King AR, Martin IL, Melville KA (1974) Reversal learning enhanced by lysergic acid diethylamide (LSD): concomitant rise in brain 5-hydroxytryptamine levels. Br J Pharmacol 52:419–426
- Kinsey AM, Wainwright A, Heavens R, Sirinathsinghji DJ, Oliver KR (2001) Distribution of 5-ht(5A), 5-ht(5B), 5-ht(6) and 5-HT(7) receptor mRNAs in the rat brain. Brain Res Mol Brain Res 88:194–198
- Klempin F, Babu H, De Pietri TD, Alarcon E, Fabel K, Kempermann G (2010) Oppositional effects of serotonin receptors 5-HT1a, 2, and 2c in the regulation of adult hippocampal neurogenesis. Front Mol Neurosci 3:1–11. Art No 14
- Koenig J, Cosquer B, Cassel JC (2008) Activation of septal 5-HT1A receptors alters spatial memory encoding, interferes with consolidation, but does not affect retrieval in rats subjected to a watermaze task. Hippocampus 18:99–118
- Ledoux J (2003) The emotional brain, fear, and the amygdala. Cell Mol Neurobiol 23:727–738
- Luttgen M, Ove OS, Meister B (2004) Chemical identity of 5-HT2A receptor immunoreactive neurons of the rat septal complex and dorsal hippocampus. Brain Res 1010:156–165



- Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20:9104–9110
- McKenna DJ, Repke DB, Lo L, Peroutka SJ (1990) Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. Neuropharmacology 29:193–198
- McNish KA, Gewirtz JC, Davis M (1997) Evidence of contextual fear after lesions of the hippocampus: a disruption of freezing but not fear-potentiated startle. J Neurosci 17:9353–9360
- Moreno FA, Wiegand CB, Taitano EK, Delgado PL (2006) Safety, tolerability, and efficacy of psilocybin in 9 patients with obsessive-compulsive disorder. J Clin Psychiatry 67:1735–1740
- Morilak DA, Garlow SJ, Ciaranello RD (1993) Immunocytochemical localization and description of neurons expressing serotonin2 receptors in the rat brain. Neuroscience 54:701–717
- Morilak DA, Somogyi P, Lujan-Miras R, Ciaranello RD (1994) Neurons expressing 5-HT2 receptors in the rat brain: neurochemical identification of cell types by immunocytochemistry. Neuropsychopharmacology 11:157–166
- Morrow BA, Elsworth JD, Rasmusson AM, Roth RH (1999) The role of mesoprefrontal dopamine neurons in the acquisition and expression of conditioned fear in the rat. Neuroscience 92:553-564
- Nibuya M, Morinobu S, Duman RS (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 15:7539–7547
- Nibuya M, Nestler EJ, Duman RS (1996) Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 16:2365–2372
- Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS (1999) Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. J Neurobiol 39:569–578
- Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, Teng KK, Yung WH, Hempstead BL, Lu B (2004) Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 306:487–491
- Passie T, Seifert J, Schneider U, Emrich HM (2002) The pharmacology of psilocybin. Addict Biol 7:357–364
- Pompeiano M, Palacios JM, Mengod G (1994) Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. Brain Res Mol Brain Res 23:163–178
- Sarnyai Z, Sibille EL, Pavlides C, Fenster RJ, McEwen BS, Toth M (2000) Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin(1A) receptors. Proc Natl Acad Sci USA 97:14731–14736
- Shen RY, Andrade R (1998) 5-Hydroxytryptamine2 receptor facilitates GABAergic neurotransmission in rat hippocampus. J Pharmacol Exp Ther 285:805–812
- Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E (2001) Neurogenesis in the adult is involved in the formation of trace memories. Nature 410:372–376

- Shors TJ, Townsend DA, Zhao M, Kozorovitskiy Y, Gould E (2002) Neurogenesis may relate to some but not all types of hippocampal-dependent learning. Hippocampus 12:578–584
- Tecott LH, Maricq AV, Julius D (1993) Nervous system distribution of the serotonin 5-HT3 receptor mRNA. Proc Natl Acad Sci USA 90:1430–1434
- Tyler WJ, Pozzo-Miller LD (2001) BDNF enhances quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses. J Neurosci 21:4249–4258
- Tyler WJ, Pozzo-Miller L (2003) Miniature synaptic transmission and BDNF modulate dendritic spine growth and form in rat CA1 neurones. J Physiol 553:497–509
- Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD (2002) From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. Learn Mem 9:224–237
- Vaidya VA, Marek GJ, Aghajanian GK, Duman RS (1997) 5-HT2A receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. J Neurosci 17:2785–2795
- Van PH, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2:266–270
- Van PH, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH (2002) Functional neurogenesis in the adult hippocampus. Nature 415:1030–1034
- Vazquez-Borsetti P, Cortes R, Artigas F (2009) Pyramidal neurons in rat prefrontal cortex projecting to ventral tegmental area and dorsal raphe nucleus express 5-HT2A receptors. Cereb Cortex 19:1678–1686
- Vilaro MT, Cortes R, Gerald C, Branchek TA, Palacios JM, Mengod G (1996) Localization of 5-HT4 receptor mRNA in rat brain by in situ hybridization histochemistry. Brain Res Mol Brain Res 43:356–360
- Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Babler A, Vogel H, Hell D (1998) Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. NeuroReport 9:3897–3902
- Vollenweider FX, Vontobel P, Hell D, Leenders KL (1999) 5-HT modulation of dopamine release in basal ganglia in psilocybininduced psychosis in man—a PET study with [11C]raclopride. Neuropsychopharmacology 20:424–433
- Willick ML, Kokkinidis L (1995) Cocaine enhances the expression of fear-potentiated startle: evaluation of state-dependent extinction and the shock-sensitization of acoustic startle. Behav Neurosci 109:929–938
- Young SN (2013) Single treatments that have lasting effects: some thoughts on the antidepressant effects of ketamine and botulinum toxin and the anxiolytic effect of psilocybin. J Psychiatry Neurosci 38:78–83

