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A single administration of the hallucinogen, 4-acetoxy-dimethyltryptamine, prevents the shift to a drug-dependent state and the expression of withdrawal aversions in rodents

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Abstract

Despite several studies suggesting the therapeutic use of 5-hydroxytryptamine receptors type 2A (5-HT_{2A}) agonists in the treatment of substance use disorders, the neurobiological basis accounting for such effects are still unknown. It has been observed that chronic exposure to drugs of abuse produces molecular and cellular adaptations in ventral tegmental area (VTA) neurons, mediated by brain-derived neurotrophic factor (BDNF). These BDNF-induced adaptations in the VTA are associated with the establishment of aversive withdrawal motivation that leads to a drug-dependent state. Growing evidence suggests that 5-HT_{2A} receptor signaling can regulate the expression of BDNF in the brain. In this study, we observed that a single systemic or intra-VTA administration of a 5-HT_{2A} agonist in rats and mice blocks both the aversive conditioned response to drug withdrawal and the mechanism responsible for switching from a drug-naive to a drug-dependent motivational system. Our results suggest that 5-HT_{2A} agonists could be used as therapeutic agents to reverse a drug dependent state, as well as inhibiting the aversive effects produced by drug withdrawal.

Introduction

Drug addiction is a chronically relapsing disorder involving substance dependence, loss of control, withdrawal, and serious long-term consequences such as physical and psychological disorders (Leshner, 1997). Addiction to substances of abuse, including nicotine, alcohol, and illicit drugs, contributes to more than 10% of all deaths worldwide (World Health Organization, 2011). Furthermore, approximately two-thirds of drug addicts who attempt to quit will relapse (NIDA, 2008), highlighting the urgent need to develop novel and more effective treatments.

Drug addiction is a pathological state that involves a complex interaction among cognitive, emotional, and motivational processes with a substance of abuse (Leshner, 1997). Recent evidence shows that neurobiological adaptations in brain reward circuitry occur after substance abuse, leading to the aversive withdrawal experience, compulsive drug consumption, and relapse (Koob & Volkow, 2010; Grieder *et al.*, 2014; Vargas-Perez *et al.*, 2014). Furthermore, it has been observed that the transition from a nondependent to a drug-dependent motivational state involves increased activity of brain-derived neurotrophic factor (BDNF) in the ventral tegmental area (VTA) of the brain reward system (Vargas-Perez *et al.*, 2009a,b). These BDNF-mediated adaptations in the brain reward circuit are necessary and sufficient to set up aversive motivational mechanisms in response to drug withdrawal and, in turn, produce and maintain a state of dependency (Grieder *et al.*, 2014; Vargas-Perez *et al.*, 2014).

Recent research suggests that increased levels of BDNF in the VTA induce the transition from a drug-naive to an opiate-dependent and withdrawn state, as a consequence of switching the neuronal substrates of drug motivation from a dopamine-independent motivational system involving the brainstem tegmental pedunculopontine nucleus (TPP), to a dopamine-dependent pathway (Vargas-Perez *et al.*, 2009b). This transition is associated with a change in gamma-aminobutyric acid type A ($GABA_A$) receptor activity on VTA GABA neurons: from hyperpolarizing (inhibitory) in naive animals,

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to depolarizing (excitatory) in drug-deprived animals or in drug-naive animals treated with intra-VTA BDNF (Vargas-Perez *et al.*, 2009b; Ting-A-Kee *et al.*, 2013). Accordingly, the disruption of intra-VTA BDNF signaling by means of selective knock down of tropomyosin-receptor-kinase type B (TrkB) receptor expression in the VTA, blocks this shift from inhibitory to excitatory $GABA_A$ receptor signaling, and keeps animals in a drug naive-like motivational state (Vargas-Perez *et al.*, 2014). Therefore, the manipulation of intra-VTA BDNF signaling constitutes a promising target for novel addiction treatments.

It has been suggested that the serotonin (5-Hydroxytryptamine, 5-HT) neurotransmitter system regulates GABA neurons in the VTA (Bankson & Yamamoto, 2004). 5-HT has long been implicated in the modulation of aversive motivation (Cools *et al.*, 2008) and the transition to, and maintenance of, drug addiction (Müller & Homberg, 2015). Furthermore, the 5-HT receptors type 2A ($5\text{-}HT_{2A}$), which are expressed on VTA dopaminergic and GABAergic neurons (Doherty & Pickel, 2000; Nocjar *et al.*, 2002), differentially regulates the expression of BDNF in the brain (Vaidya *et al.*, 1999). It has been suggested that $5\text{-}HT_{2A}$ agonists could reduce the expression of BDNF in GABAergic interneurons (Vaidya *et al.*, 1999; Hamon & Blier, 2013). Thus, we hypothesized that the administration of a $5\text{-}HT_{2A}$ agonist would block both the aversive conditioned response to drug withdrawal and the development of the drug-dependent state.

To test this hypothesis, we used the non-selective $5\text{-}HT_{2A}$ agonist 4-Acetoxy-Dimethyltryptamine (4-AcO-DMT) in chronic opiate-, chronic nicotine- or intra-VTA BDNF-treated but previously drug naive rodents. We observed that the systemic or intra-VTA administration of 4-AcO-DMT prevented the switch from a drug-naive to a drug-dependent motivational state, and also blocked the conditioned aversive response to drug withdrawal.

Materials and methods

Animals

Male Wistar rats (Charles River) weighing 350–500 g and male C57BL/6 mice (Charles River) weighing 25–30 g were housed individually in Plexiglas cages in a room maintained at 22 °C and lit from 7:00 A.M. to 7:00 P.M. Rats and mice were given food and water *ad libitum* throughout the experiment. The University of Toronto Animal Care Committee approved all experiments in accordance with the Canadian Council on Animal Care guidelines.

Drugs and microinjection procedure

BDNF (Sigma) was dissolved in phosphate-buffered saline (pH adjusted to 7.4) and infused intra-VTA. Alpha-flupenthixol (Sigma) and morphine sulfate (Almat Pharmachem) were dissolved in 0.9% saline and injected intra-peritoneally (i.p.). Diacetylmorphine hydrochloride (heroin; Almat Pharmachem) was dissolved in 0.9% saline and injected subcutaneously (s.c.). Nicotine hydrogen tartrate salt (Sigma) was dissolved in saline (pH 7.4) and administered via osmotic minipumps (chronic nicotine, minipump model 1002, Alzet) or subcutaneous (s.c.) injection (acute nicotine). 4-AcO-DMT (Avanztec) was dissolved in 0.9% saline and injected i.p., or dissolved in phosphate-buffered saline (pH 7.4) for intra-VTA infusions.

VTA microinjections (0.5 μL volume per infusion) were performed over 1 min. Injectors were left in place for a further 1 min to ensure adequate diffusion from the injector tip.

Blockade of dopaminergic system

The dopaminergic system was blocked with systemic administration of the neuroleptic alpha-flupenthixol (0.8 mg/kg, i.p.) which antagonizes both D1 and D2 type receptors, injected 2.5 h before conditioning in rats and 1 h before conditioning in mice.

Induction of drug dependence

To induce opiate dependence in rats, animals received 0.5 mg/kg s.c. injections of heroin daily for 5 days prior to conditioning until the end of the conditioning schedule (Laviolette *et al.*, 2004). Opiate dependent and withdrawal animals were conditioned for the motivational effects of morphine 21 h after their last heroin injection. During conditioning days, this dose of heroin was administered as a maintenance dose 3.25 h after the termination of conditioning sessions.

A group of mice was induced to a drug dependent-like effect by means of a single intra-VTA BDNF (0.25 μg) infusion (Vargas-Perez *et al.*, 2009a,b; Ting-A-Kee *et al.*, 2013), Mice were left to recover for 7 days before they were used in behavioral experiments.

Mice were made nicotine dependent by the implantation of subcutaneous osmotic mini pumps (Model 1002; Alzet) containing nicotine (7 mg/kg/day) dissolved in saline solution (pH 7.4; Grieder *et al.*, 2014). The pumps delivered nicotine for 12 days and then experiments were performed 8 h following surgical removal of the pumps, when the animal was experiencing peak somatic and motivational withdrawal (Grieder *et al.*, 2010).

Surgery and histology

Animals were anesthetized with inhaled isoflurane (3–5% to induce anesthesia and 2–3% to maintain anesthesia) and placed in a stereotaxic device. For microinfusion cannulae in rats, 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA, USA) were bilaterally implanted 2 mm dorsal to the VTA at a 10° angle using the following coordinates relative to bregma: AP, −5.6 mm; ML, ±2.3 mm and DV, −7.8 mm from the dural surface. At least 2 weeks were allowed for post-surgical recovery preceding behavioral training.

Infusions of BDNF were performed bilaterally by injecting 0.25 μL total of a 0.5 μg/μL concentration of BDNF dissolved in phosphate buffered saline (PBS; pH = 7.4). Microinfusions were performed with a 5 μL Hamilton microsyringe (VWR), needle blunt point style (33-gauge), over a 5 min period after which the injector was left in place for an additional 1 min to allow for diffusion of the solution from the injector tip. The VTA injection coordinates, from bregma, were: AP, −3.0 mm; ML, −0.6 mm; and DV, −4.1 mm from the dural surface. Animals were given 1 week of recovery time before conditioning.

At the end of experiments, animals were deeply anesthetized with sodium pentobarbital (Somnotol, 0.8 mL/kg, i.p.) and were perfused transcardially with 0.9% saline followed by 10% formalin. Brains were rapidly removed and stored for 12 h in 25% sucrose in a 10% formalin solution. Brains were then flash-frozen at −70 °C, sliced in a freezing microtome at −20 °C into 40 μm-thick sections, and mounted on gelatin-coated slides. Sections from the VTA were processed for cresyl violet staining and subsequently examined by light microscopy to ensure correct placement of cannulae.

For the implantation of osmotic minipumps in mice, animals were anesthetized by inhalation of 5% isofluorane in oxygen (1–2% maintenance), an incision made between the scapulae, and the minipump

placed subcutaneously between the scapulae parallel to the spine. After minipump implantation, the surgical incision was sutured and treated with antibiotic cream.

Pretreatment with 4-AcO-DMT

4-AcO-DMT was used to test the effects of systemic (4 mg/kg in rats, Walters *et al.*, 1978; File, 1977; 10 mg/kg in mice, Shan & Hedden, 1978) or intra-VTA (0.25 μg) acute administration of 5-HT_{2A} agonists in subjects experiencing an aversive motivational state produced by drug withdrawal or the development of a drug-dependent state.

Intra-VTA Infusions of 4-AcO-DMT were performed bilaterally by injecting 0.25 μL total (Smyth *et al.*, 1998) of a 0.5 μg/μL concentration of BDNF dissolved in phosphate buffered saline (PBS; pH = 7.4). Sham controls received bilateral injections of PBS alone. Microinfusions were performed with an internal cannulae (injector, Plastics One, Roanoke, VA, USA), blunt point style (28-gauge), over a 5 min period after which the injector was left in place for an additional 1 min to allow for diffusion of the solution from the injector tip.

Experimental design

The first set of experiments was designed to test if a single 4-AcO-DMT administration can switch a subject from a dopamine mediated opiate dependent state back to a nondependent (non dopamine mediated) state. Rats were made opiate dependent by administering daily heroin injections by 5 days; a group of mice were intra-VTA infused with BDNF (0.25 μg) to induce a dependent-like state. Dependent rats were pretreated with a single administration of 4-AcO-DMT (4 mg/kg i.p., or 0.25 μg intra-VTA) 1 day before conditioning sessions started. Intra-VTA BDNF-infused mice were pretreated with a single administration of 4-AcO-DMT (10 mg/kg i.p.) 1 day before conditioning sessions started. Place preference conditioning and testing were performed to observe whether opiate reward was blocked by alpha-flupenthixol administration. Thus, animals received a single dose of 4-AcO-DMT prior to morphine conditioning and alpha-flupenthixol administration (Fig. 1a and b).

The second set of experiments was aimed to test the effects of pretreatment with a single i.p., injection of 4-AcO-DMT on the development of a drug-withdrawal induced aversive motivational state in opiate dependent animals. Rats were made opiate dependent by administering daily heroin injections by 5 days. Opiate dependent rats were pretreated with a single administration of 4-AcO-DMT (4 mg/kg i.p.) 1 day before conditioning sessions started. Place preference conditioning and testing were performed to test the motivational effects of spontaneous withdrawal from opiates in dependent rats and naloxone-precipitated withdrawal (5 mg/kg) in opiate dependent rats (Fig. 1c and d).

In the third set of experiments, we examined whether 4-AcO-DMT administration would prevent the conditioned motivational responses to nicotine in nicotine-dependent mice. A group of nicotine naive mice were pretreated with a single administration of 4-AcO-DMT (10 mg/kg i.p.) 1 day before minipump implantation (Early group). A group of nicotine dependent mice received a single injection of 4-AcO-DMT (10 mg/kg i.p.) just after minipump removal (Late group). There was a group that received two injections (1 day before minipump implantation and just after minipump removal = Early plus Late group) of 4-AcO-DMT (10 mg/kg i.p.) before conditioning. These groups were tested for the motivational effects of nicotine withdrawal. Finally, a group of nicotine naive mice were pretreated with a single administration of 4-AcO-DMT (10 mg/kg i.p.) 1 day before minipump implantation (Early) to observe the effects of nicotine administration in nicotine dependent animals (Fig. 1e and f).

Place preference conditioning

Mice and rats were conditioned as described previously (Vargas-Perez *et al.*, 2014). Briefly, mice were conditioned in an apparatus consisting of two different environments measuring 15 × 15 × 15 cm (Med Associates, SOF-700RA-25). One environment was black with a metal rod floor and the other was white with a wire mesh floor. These conditioning environments are motivationally balanced such that animals show no initial preference for either environment before conditioning (Grieder *et al.*, 2010). An intermediate gray area housed a removable partition. Each cage was cleaned between animals and each group was fully counterbalanced. During preference testing, the dividing partition was removed and mice were given free access to both environments. A single 10 min preference-testing session was performed at least 2 days after the last conditioning day, when mice were drug- and withdrawal-free.

Behavioral testing for rats consisted of three phases: pre-exposure, conditioning, and testing. The pre-exposure phase comprised a single 20-min session in separate boxes (41 × 41 × 38 cm) painted gray with a gray floor. Conditioning took place in one of two distinct environments (41 × 41 × 38 cm), which differed in color, texture, and smell. One environment was white, with a wire mesh floor. The other environment was black, with a smooth Plexiglas floor that was wiped down with a 12% acetic acid solution before each conditioning session. These conditioning environments are motivationally balanced such that animals show no initial preference for either environment before conditioning (Laviolette *et al.*, 2004). During testing, each rat was placed in the neutral gray zone (41 × 10 cm) that separated the two compartments and was allowed to explore both environments freely for a period of 10 min.

The conditioning phase consisted of four to eight sessions of 40 min each for rats. The conditioning phase in BDNF-treated mice consisted of eight sessions of 15 min, and in nicotine-treated mice conditioning consisted of a single session of 1 h. All place conditioning and tests were performed between 08:30 and 19:00 h.

Procedure B (both drug and withdrawal pairing). In this procedure, animals were exposed to both conditioning environments in a fully counterbalanced order. Thus, each animal experienced the effects of a drug in one environment and the lack of drug, just the vehicle saline injection (the effects of withdrawal), in the other environment. All experimental groups received four drug- environment and four withdrawal-environment conditioning sessions over 8 consecutive days. Conditioning was performed immediately after drug or saline injection. Testing was performed drug-free, at least 2 days after the final conditioning session. The difference score for each animal was calculated by subtracting the time spent in the withdrawal-paired environment from the time spent in the drug-paired environment. Under this place conditioning procedure we assessed the motivational effects of i.p., morphine (10 mg/kg in mice and rats) and naloxone-precipitated withdrawal (5 mg/kg in rats). In dependent rats and BDNF-treated mice, morphine was administrated after the blockade of the dopaminergic system with the neuroleptic alpha-flupenthixol (0.8 mg/kg, i.p.).

Procedure D (drug only) was a modified place conditioning procedure used to measure the motivational effects of drug administration on its own. Conditioning took place in only the drug-paired environment of the place conditioning apparatus. Each animal was injected

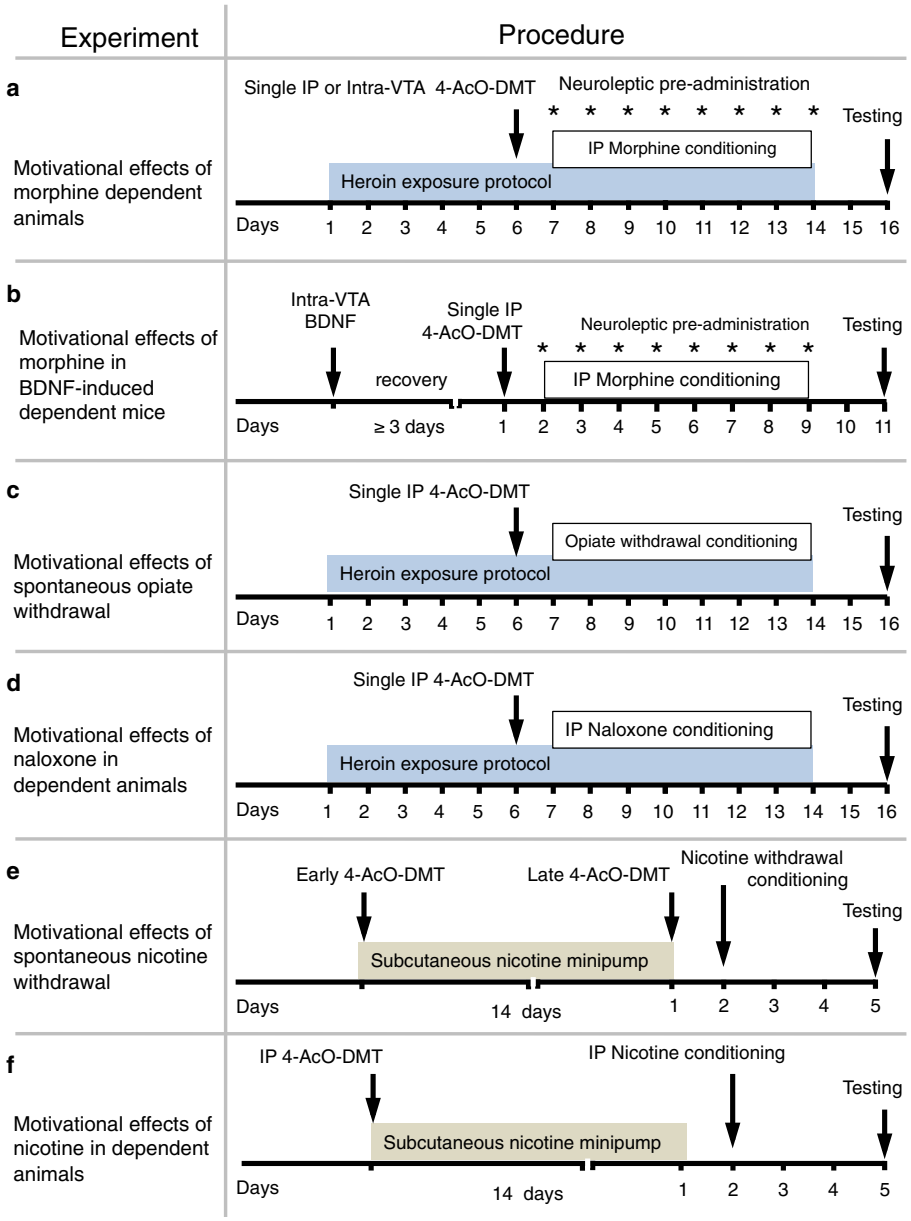

FIG. 1. Schematic descriptions of the experimental timelines. (a and b) First set of experiments. (c and d) Second set of experiments. (e and f) Third set of experiments. [Colour figure can be viewed at wileyonlinelibrary.com].

with drug and confined to one of the environments immediately after drug administration. On the alternate day, the animal was injected with saline solution and immediately put back in their home cage. Since the animals were never allowed to experience withdrawal in the other compartment of the place conditioning apparatus, this procedure is thought to measure the conditioned motivational effects for the drug itself (Bechara *et al.*, 1995). After testing, the difference score for each animal was calculated by subtracting the time spent in the non-paired environment from the time spent in the drug-paired environment. This procedure was used to assess the effects of pretreatment with a single 4-AcO-DMT administration in chronic nicotine dependent and withdrawn animals. During nicotine withdrawal, 8 h after a nicotine-containing minipump was removed, nicotine (1.5 mg/kg) or its control saline was administered and the animals were immediately confined to one of the conditioning environments for 1 h. In this way, the motivational effects of chronic nicotine in a dependent animal were assessed.

In procedure W (withdrawal only), conditioning took place in only the withdrawal-paired environment of the place conditioning apparatus. During a state of drug withdrawal, 16 h after the last injection of opiate, each animal was injected with saline vehicle and then exposed immediately to a distinct conditioning environment. As a result, one of the two compartments was paired with the absence a previously administered drug and the other was an unfamiliar, neutral environment. Animals were tested drug-free at least 2 days after the last conditioning session. The times spent in each environment were recorded over 10 min test periods. The difference score for each animal was calculated by subtracting the time spent in the non-paired environment from the time spent in the withdrawal-paired environment. Under this procedure we tested the

motivational effects of spontaneous withdrawal from opiates in dependent rats (Bechara *et al.*, 1995; Vargas-Perez *et al.*, 2007, 2009a). Procedure W also was used for nicotine dependent and withdrawn experiments in mice (Grieder *et al.*, 2010). Mice received a sham surgery in which the minipump was removed and replaced immediately, controlling for any effects of surgery. For the remainder of the day, the animal was confined to its home cage. On the conditioning day, the minipump was removed and mice were confined for 1 h to one of the conditioning environments for a single session after 8 h, when the animal was experiencing the most intense withdrawal (Grieder *et al.*, 2010).

Statistical analyses

Results were analyzed using a one-way analysis of variance (ANOVA) and Student's *t*-tests where appropriate. In all cases, a normality test and an equal variance test were performed before the ANOVA to ensure its validity. *Post hoc* Duncan's tests were used where appropriate. Data from each group are shown as a mean line, with dots representing the scores of single individuals.

Results

4-AcO-DMT switches an animal back to a drug nondependent motivational state

To examine if a single administration of 4-AcO-DMT could reverse the switch to a dopamine mediated opiate dependent motivational state, we made rats opiate dependent by administering daily heroin injections and used the B procedure to test whether opiate reward was blocked by alpha-flupenthixol administration after the single 4-AcO-DMT pretreatment. A one-way ANOVA showed a significant effect of treatment in both rats ($F_{4,39} = 8.76$, $P = 0.00005$, Fig. 2a) and mice ($F_{2,23} = 5.71$, $P = 0.01$, Fig. 2b). Opiate-dependent rats intra-VTA infused with saline and injected with systemic alpha-flupenthixol did not show any place preference ($n = 8$, $P = 0.63$), while opiate-dependent rats that received a single 4-AcO-DMT injection into the VTA showed a place preference for an environment where they received morphine administration that was not blocked by alpha-flupenthixol ($n = 8$, $P = 0.0008$). These results suggest that intra-VTA 4-AcO-DMT can switch a subject from a dopamine mediated opiate dependent state back to a nondependent (non dopamine mediated) state.

The specificity of this effect was demonstrated further by results showing opiate-dependent rats that received intracerebral 4-AcO-DMT rostral, ventral, or lateral to the VTA (misplaced cannulae, Fig. S1) did not show conditioned place preferences for the motivational effects of morphine after alpha-flupenthixol pretreatment compared to the morphine control group ($n = 8$, $P = 0.038$). In addition, a single administration of systemic 4-AcO-DMT had the same effect as intra-VTA 4-AcO-DMT, such that the place preferences induced by the motivational effects of morphine were not blocked by alpha-flupenthixol pretreatment ($n = 8$, $P = 0.85$). These results suggest that a 5-HT_{2A} agonist administered systemically can revert a drug dependent animal back to a drug nondependent motivational state.

Similar to the rat results, when mice that were in a BDNF-induced-dependent state were pretreated with alpha-flupenthixol, they did not show a conditioned place preference for the motivational effects of morphine administration ($n = 8$, $P = 0.01$). However, BDNF-induced dependent mice pretreated with a single systemic 4-AcO-DMT injection ($n = 8$, $P = 0.53$) showed conditioned place preferences for environments paired with the motivational effects of morphine, which were not blocked by alpha-flupenthixol. These results lend support to the hypothesis that a single injection of the 5-HT_{2A} agonist 4-AcO-DMT given systemically can switch the motivational state of a subject back to a 'drug-naive' state, even after chronic opiate exposure or intra-VTA BDNF administration has produced a dependent motivational state.

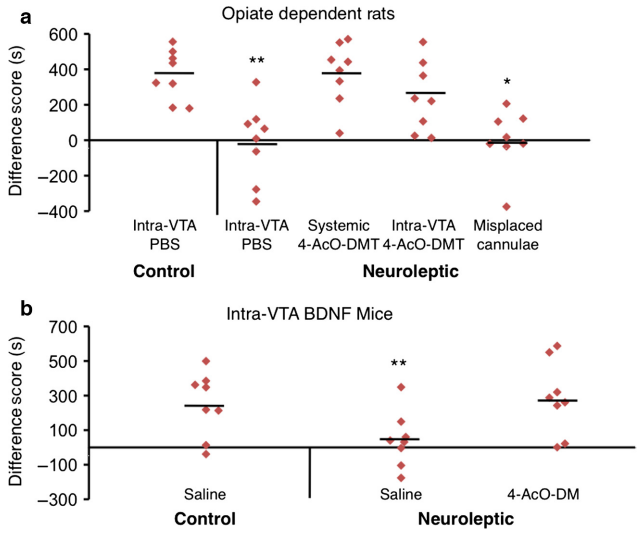

FIG. 2. 4-AcO-DMT can reverse a dependent motivational state. (a) Disruption of dopaminergic signaling with a broad spectrum neuroleptic (alpha-flupenthixol, 0.8 mg/kg) blocked the rewarding effects of morphine (10 mg/kg) in opiate dependent rats pretreated with Intra-VTA PBS $n = 8$, $P = 0.0008$, compared to rats pretreated with single i.p., (4 mg/kg, $n = 8$, $P = 0.85$, or intra-VTA (0.25 μg, $n = 8$, $P = 0.53$) administrations of 4-AcO-DMT, where neuroleptics failed to block morphine place preferences. Compared to the intra-VTA PBS control group which showed a morphine place preference, opiate dependent rats receiving administration of 4-AcO-DMT through cannulae outside of the VTA (misplaced cannulae) did not show conditioned place preferences for the motivational effects of morphine after neuroleptic pretreatment ($n = 8$, $P = 0.038$) (b) Disruption of dopaminergic signaling with a neuroleptic (alpha-flupenthixol, 0.8 mg/kg) blocked the rewarding effects of morphine (10 mg/kg) in BDNF-induced dependent mice ($n = 8$, $P = 0.01$), but did not block the rewarding effects of morphine in a group receiving a single i.p., ($n = 8$, $P = 0.53$) administration of 4-AcO-DMT (10 mg/kg, $n = 8$). Each dot represents a single individual (*$P < 0.05$, **$P < 0.01$). [Colour figure can be viewed at wileyonlinelibrary.com].

4-AcO-DMT prevents the conditioned aversive response to opiate withdrawal

The effects of pretreatment with a single i.p. injection of 4-AcO-DMT on the development of a drug-withdrawal induced aversive motivational state were tested using place conditioning. Using the W procedure in opiate-dependent rats, *t*-tests showed that the conditioned place aversion associated with opiate withdrawal was blocked in 4-AcO-DMT pretreated rats ($t_{14} = 2.83$, $n = 8$, $P = 0.01$, Fig. 3a) compared with saline pretreated rats (which show conditioned place aversions to an environment paired with 16 h of opiate withdrawal). However, using the B procedure in dependent rats, the place aversions induced by the administration of the opioid antagonist naloxone (that are seen in both opiate-naïve as well as opiate-dependent animals) were not blocked in 4-AcO-DMT pretreated rats ($t_{14} = 0.38$, $n = 8$, $P = 0.70$, Fig. 3a), compared with control saline pretreated rats. These results suggest that similar to what occurs with the blockade of BDNF signaling (Vargas-Perez *et al.*, 2014), 4-AcO-DMT administration is able to prevent the development of the

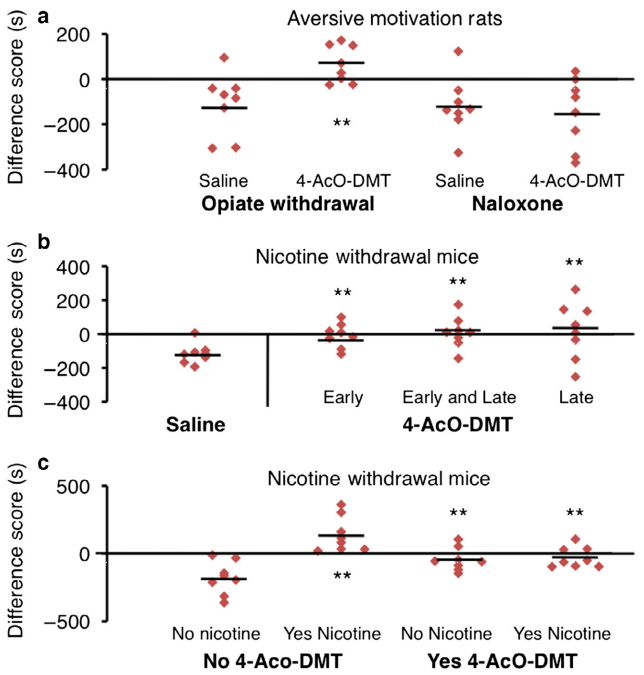

FIG. 3. 4-AcO-DMT blocked the aversive motivation produced from spontaneous drug withdrawal. (a) Pretreatment with a single administration of 4-AcO-DMT (4 mg/kg) blocked the place aversion for 16 h of abstinence from morphine (3 mg/kg) in dependent rats (n = 8, P = 0.01), but not in opiate dependent animals pretreated with saline (n = 8). In contrast, a single administration of 4-AcO-DMT did not block naloxone-conditioned (5 mg/kg) place aversions (n = 8, P = 0.70). (b) Compared with saline pretreated animals, pretreatment with 4-AcO-DMT (10 mg/kg), before (Early) nicotine minipump implantation (7 mg/kg/day, free base) and/or after (Late) minipump removal, blocked the place aversions for environments paired at 8 h after nicotine withdrawal in mice (P = 0.01). (c) The Early pretreatment with a single administration of 4-AcO-DMT (i.p., 10 mg/kg) blocked both a nicotine withdrawal aversion (P = 0.01) and the reward (P = 0.007) produced by a single dose of nicotine (1.75 mg/kg, s.c.) in nicotine dependent animals. Each dot represents a single individual (**P < 0.01). [Colour figure can be viewed at wileyonlinelibrary.com].

aversive motivational state induced by spontaneous withdrawal from opiate administration.

4-AcO-DMT prevents the conditioned motivational responses to nicotine in dependent mice

We next examined whether 4-AcO-DMT administration would prevent nicotine withdrawal aversions. Similar to opiate-dependent and withdrawn rats, when nicotine dependent and withdrawn mice were conditioned in the W procedure, a one-way ANOVA showed an effect of treatment ($F_{4,31}$ = 3.87, P = 0.01, Fig. 3b). Compared with saline pretreated animals, the pretreatment with 4-AcO-DMT before (Early, N = 8, P = 0.005) nicotine minipump implantation, after (Late, N = 8, P = 0.006) minipump removal, or at both times (Early and Late, N = 8, P = 0.01), blocked the place aversion for 8 h of nicotine withdrawal in mice. These results suggest that a single administration of 4-AcO-DMT can prevent the aversive motivational response to nicotine withdrawal.

To investigate whether 4-AcO-DMT would also affect conditioned nicotine reward in nicotine-dependent and withdrawn mice, we gave a single injection of nicotine to mice that were in nicotine withdrawal in the D procedure. A one-way ANOVA showed an effect of treatment ($F_{4,31}$ = 12.15, P = 0.00003, Fig. 3c). Control mice that received saline showed a conditioned place preference for the nicotine-paired environment (n = 8, P = 0.0001), suggesting that nicotine is rewarding in a nicotine-dependent and withdrawn animal. Pretreatment with a single injection of systemic 4-AcO-DMT prior to chronic nicotine administration blocked the expression of both the nicotine withdrawal aversion (n = 8, P = 0.01) and acute nicotine place preference (n = 8, P = 0.007), suggesting that 4-AcO-DMT administration may prevent the rewarding motivational effects of nicotine in nicotine-dependent mice by blocking the aversion to withdrawal (thus blocking negative reinforcement).

Discussion

Several lines of evidence suggest that the aversive motivational state, elicited by the withdrawal of a drug, constitutes one of the main drivers of addiction (Koob & Le Moal, 2001; Vargas-Perez *et al.*, 2007, 2009a). In particular, it has been observed that the constant cycle of drug administration required to 'escape' from the negative state of drug withdrawal produces BDNF-related neural plasticity in the VTA, which is itself necessary (Vargas-Perez *et al.*, 2014) and sufficient (Vargas-Perez *et al.*, 2009b) to develop a drug-dependent motivational state. In this study using a place preference paradigm, we observed that similar to what occurs with the blockade of BDNF signaling in the VTA (Vargas-Perez *et al.*, 2014), a single systemic or intra-VTA administration of the 5-HT_{2A} agonist 4-AcO-DMT is sufficient to prevent or reverse the switch from a drug-naive to a drug-dependent motivational state. Pretreatment of 4-AcO-DMT prevents the switch to a dopamine-dependent reward motivational system, regardless of whether this dependent state was produced by chronic administration of opiates or by intra-VTA BDNF administration. Furthermore, a single administration of 4-AcO-DMT was sufficient to block the aversive motivational effects produced by withdrawal from chronic opiate and chronic nicotine administration. However, these last effects are not related to a general loss in the ability to detect aversive events, as animals pretreated with 4-AcO-DMT were able to demonstrate conditioned place aversions induced by the opioid receptor antagonist naloxone (which produces aversions in both the drug-naïve and drug-dependent states; Vargas-Perez *et al.*, 2014).

Remarkably, a single 4-AcO-DMT administration had a protective effect against the development of aversive motivation – even if administered prior to chronic exposure to the drug of abuse. We observed that drug-naive animals pretreated with 4-AcO-DMT did not demonstrate withdrawal aversions after chronic nicotine exposure. Furthermore, the capacity of acute nicotine administrations to change from aversive (in drug-naive animals) to rewarding (in drug-dependent animals) was blocked. Acute nicotine was no longer able to relieve aversion in nicotine-withdrawn animals if they were pretreated with 4-AcO-DMT. These results suggest that 4-AcO-DMT prevented the development of nicotine withdrawal in these animals. These results are in line with recent findings in human populations suggesting that 5-HT_{2A} agonist administration is a potentially efficacious adjunct to current smoking cessation treatment models (Johnson *et al.*, 2014).

The precise VTA adaptations induced by 4-AcO-DMT that could lead to the longer term outcomes of preventing and reversing the development of a drug dependent motivation state currently are unclear. It is known that BDNF and 5-HT_{2A} signaling are closely intertwined, both 5-HT and TrkB signaling are involved in the modulation of longer term neuronal survival and plasticity (Mattson *et al.*, 2004). It is unknown if these effects are caused by 5-HT2A/TrkB direct interactions or downstream 5-HT2A signaling/BNDF signaling interactions. Additionally, some reports suggest that 5-HT_{2A} receptor agonists are able to down-regulate cyclic adenosine monophosphate (cAMP) production, which could leads to a decrease in BDNF transcription in GABAergic

interneurons (Vaidya *et al.*, 1997; Martinowich & Lu, 2008). The mechanism that may underlies this potential neural and cell type dependent effects are still unknown. However, this neuronal change, in addition to preventing and/or reversing a depolarizing GABAergic response, could cause a substantial change in the inhibitory GABAergic signaling over VTA dopaminergic neurons, thereby altering considerably the activity of the mesolimbic dopaminergic system and perhaps producing a robust and long-lasting positive motivational state (White, 1996; Ting-A-Kee *et al.*, 2013).

It has been observed that 5-HT_{2A} is able to regulate aberrant glutamatergic activity, activity which is suggested to play an important role in the neuronal changes associated with the development and progression of aversive motivational states that can lead to depression and addiction (Marek *et al.*, 2000). 5-HT_{2A} receptors are capable of forming heteromeric complexes with metabotropic glutamate (mGlu) receptors. 5-HT_{2A} and mGlu receptors physiologically bind each other, leading to reciprocal regulation of their functions (González-Maeso *et al.*, 2008; Moreno *et al.*, 2012). Also, 5-HT signaling can interact with N-Methyl-D-aspartate (NMDA) receptors, regulating neuronal firing (Yuen *et al.*, 2008; Zhong *et al.*, 2008). Indeed, it has been observed that the regulation of glutamatergic activity with the non-competitive NMDA antagonist ketamine causes rapid relief from aversive motivational symptoms related to depression in as quickly as a couple of hours (Lapidus *et al.*, 2013; Niciu *et al.*, 2014). However, possibly due to dynamic trafficking of synaptic NMDA receptors, the duration of this effect is limited to less than a couple of weeks and in the best of best of cases to 2 months (Lapidus *et al.*, 2013; Niciu *et al.*, 2014). The potential therapeutic impact of 5-HT_{2A} receptor agonists may last significantly longer, over 6 months and perhaps, even years (Griffiths *et al.*, 2008; Johnson *et al.*, 2008, 2014). This may suggest an important role for 5-HT_{2A} receptors in the long-term regulation of synaptic plasticity.

Taken together, our results suggest that a single administration of a 5-HT_{2A} agonist can prevent and reverse the VTA neural modifications controlling the switch from a drug-nondependent to a drug-dependent motivational state, including the production of aversive withdrawal motivation. A single administration of 4-AcO-DMT can reverse the switch to a dependent motivational state, possibly by preventing the up-regulation of BDNF and thus the key neural changes in the VTA that mediate the aversive effects produced by drug withdrawal and the development of a drug-dependent state. However, more research is needed to conclude that the behavioral effects observed were caused solely by regulation of BDNF and 5-HT_{2A} receptor activation.

Our results are in agreement with pre-clinical studies suggesting that the use of 5-HT_{2A} agonists may be useful as therapeutic agents in the treatment of addiction (de Rios *et al.*, 2002; Thomas *et al.*, 2013; Johnson *et al.*, 2014; Loizaga-Velder & Verres, 2014). Indeed, the findings are consistent with data showing that administration of 5-HT_{2A} receptor agonists is negatively correlated with the development of addictive behavior in human populations (Gable, 2007). Furthermore, 5-HT_{2A} receptor agonists have been reported to be therapeutically useful (Vargas-Perez & Doblin, 2013) in producing long-lasting relief from the negative symptoms associated with aversive motivational states such depression (de Lima Osório *et al.*, 2015) and addiction (Thomas *et al.*, 2013; Loizaga-Velder & Verres, 2014).

Supporting Information

Additional supporting information can be found in the online version of this article:

Fig. S1. Schematic representation of the misplaced cannulae for the 4-AcO-DMT infusions in rats: cannulae that were rostral, ventral, or lateral to the VTA. Xs mark the tips of representative misplaced cannulae. The coordinates are relative to bregma. (CP, cerebral peduncle; SN, substantia nigra; VTA, ventral tegmental area.)

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Conflict of interest

The authors declare no competing financial interests.

Author contributions

H.V.-P., T.E.G., and D.v.d.K designed the experiments. The data were collected and Analyzed by H.V.-P., T.E.G., R.T.-A.-K., G.M.-B., and M.C. H.V.-P., and D.v.d.K. wrote the paper.

Data accessibility

On acceptance for publication the raw data will be deposited on the website figshare.com.

Abbreviations

4-AcO-DMT, 4-Acetoxy-Dimethyltryptamine; 5-HT_{2A}, 5-Hydroxytryptamine Receptors Type 2A; 5-HT, 5-Hydroxytryptamine, Serotonin; AP, Anterior-Posterior; BDNF, Brain-Derived Neurotrophic Factor; cAMP, Cyclic Adenosine Monophosphate; CP, Cerebral Peduncle; D1, Dopaminergic type 1; D2, Dopaminergic type 2; DA, Dopamine; DV, Dorsal-Ventral; $GABA_A$, Gamma-Aminobutyric Acid Type A Receptor; GABA, Gamma-Aminobutyric Acid; i.p., Intra-Peritoneally; mGlu, Metabotropic Glutamate; ML, Middle-Lateral; NAcc, Nucleus Accumbens; NMDA, N-Methyl-D-aspartate; PBS, Phosphate Buffered Saline; s.c., Subcutaneously; SN, Substantia Nigra; TPP, Tegmental Pedunculopontine Nucleus; TrkB, Tropomyosin-Receptor-Kinase Type B; VTA, Ventral Tegmental Area; μ, Mu opiod receptor.

References

Bankson, M.G. & Yamamoto, B.K. (2004) Serotonin-GABA interactions modulate MDMA-induced mesolimbic dopamine release. *J. Neurochem.*, **91**, 852–859.

Bechara, A., Nader, K. & van der Kooy, D. (1995) Neurobiology of withdrawal motivation: evidence for two separate aversive effects produced in morphine-naive versus morphine-dependent rats by both naloxone and spontaneous withdrawal. *Behav. Neurosci.*, **109**, 91–105.

Cools, R., Roberts, A.C. & Robbins, T.W. (2008) Serotoninergic regulation of emotional and behavioural control processes. *Trends Cogn. Sci.*, **12**, 31–40.

Doherty, M.D. & Pickel, V.M. (2000) Ultrastructural localization of the serotonin 2A receptor in dopaminergic neurons in the ventral tegmental area. *Brain Res.*, **864**, 176–185.

File, S.E. (1977) Effects of N, N-dimethyltryptamine on behavioural habituation in rat. *Pharmacol. Biochem. Be.*, **6**, 163–168.

Gable, R.S. (2007) Risk assessment of ritual use of oral dimethyltryptamine (DMT) and harmala alkaloids. *Addiction*, **102**, 24–34.

González-Maeso, J., Ang, R.L., Yuen, T., Chan, P., Weisstaub, N.V., López-Giménez, J.F., Zhou, M., Okawa, Y. *et al.* (2008) Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature*, **452**, 93–97.

Grieder, T.E., Herman, M.A., Contet, C., Tan, L.A., Vargas-Perez, H., Cohen, A., Chwalek, M., Maal-Bared, G. *et al.* (2014) VTA CRF neurons mediate the aversive effects of nicotine withdrawal and promote intake escalation. *Nat. Neurosci.*, **17**, 1751–1758.

Grieder, T.E., Sellings, L.H., Vargas-Perez, H., Ting-A-Kee, R., Siu, E.C., Tyndale, R.F. & van der Kooy, D. (2010) Dopaminergic signaling mediates the motivational response underlying the opponent process to chronic but not acute nicotine. *Neuropsychopharmacology*, **35**, 943–954.

Griffiths, R., Richards, W., Johnson, M., McCann, U. & Jesse, R. (2008) Mystical-type experiences occasioned by psilocybin mediate the attribution of personal meaning and spiritual significance 14 months later. *J. Psychopharmacol.*, **22**, 621–632.

Hamon, M. & Blier, P. (2013) Monoamine neurocircuitry in depression and strategies for new treatments. *Prog. Neuropsychopharmacol. Biol. Psychiat.*, **45**, 54–63.

Johnson, M.W., Richards, W. & Griffiths, R.R. (2008) Human hallucinogen research: guidelines for safety. *J. Psychopharmacol.*, **22**, 603–620.

Johnson, M.W., Garcia-Romeu, A., Cosimano, M.P. & Griffiths, R.R. (2014) Pilot study of the 5-HT2AR agonist psilocybin in the treatment of tobacco addiction. *J. Psychopharmacol.*, **28**, 983–992.

Koob, G.F. & Le Moal, M. (2001) Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacol.*, **24**, 97–129.

Koob, G.F. & Volkow, N.D. (2010) Neurocircuitry of addiction. *Neuropsychopharmacol.*, **35**, 217–238.

Lapidus, K.A., Soleimani, L. & Murrough, J.W. (2013) Novel glutamatergic drugs for the treatment of mood disorders. *Neuropsych. Dis. Treat.*, **9**, 1101–1112.

Laviolette, S.R., Gallegos, R.A., Henriksen, S.J. & van der Kooy, D. (2004) Opiate state controls bi-directional reward signaling via GABAA receptors in the ventral tegmental area. *Nat. Neurosci.*, **7**, 160–169.

Leshner, A.I. (1997) Addiction is a brain disease, and it matters. *Science*, **278**, 45–47.

de Lima Osório, F., Sanches, R.F., Macedo, L.R., Dos Santos, R.G., Maia-de-Oliveira, J.P., Wichert-Ana, L., de Araujo, D.B., Riba, J. *et al.* (2015) Antidepressant effects of a single dose of ayahuasca in patients with recurrent depression: a preliminary report. *Rev. Bras. Psiquiatr.*, **37**, 13–20.

Loizaga-Velder, A. & Verres, R. (2014) Therapeutic effects of ritual ayahuasca use in the treatment of substance dependence–qualitative results. *J. Psychoactive Drugs*, **46**, 63–72.

Marek, G.J., Wright, R.A., Schoepp, D.D., Monn, J.A. & Aghajanian, G.K. (2000) Physiological antagonism between 5-hydroxytryptamine(2A) and group II metabotropic glutamate receptors in prefrontal cortex. *J. Pharmacol. Exp. Ther.*, **292**, 76–87.

Martinowich, K. & Lu, B. (2008) Interaction between BDNF and serotonin: role in mood disorders. *Neuropsychopharmacol.*, **33**, 73–83.

Mattson, M.P., Maudsley, S. & Martin, B. (2004) BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci.*, **27**, 589–594.

Moreno, J.L., Muguruza, C., Umali, A., Mortillo, S., Holloway, T., Pilar-Cuéllar, F., Mocci, G., Seto, J. *et al.* (2012) Identification of three residues essential for 5-hydroxytryptamine 2A-metabotropic glutamate 2 (5-HT2A·mGlu2) receptor heteromerization and its psychoactive behavioral function. *J. Biol. Chem.*, **287**, 44301–44319.

Müller, C.P. & Homberg, J.R. (2015) The role of serotonin in drug use and addiction. *Behav. Brain Res.*, **277**, 146–192.

NIDA. (2008) NIDA InfoFacts: Understanding Drug Abuse and Addiction. Available http://www.drugabuse.gov/infofacts/understand.html

Niciu, M.J., Henter, I.D., Luckenbaugh, D.A., Zarate, C.A. & Charney, D.S. (2014) Glutamate receptor antagonists as fast-acting therapeutic alternatives for the treatment of depression: ketamine and other compounds. *Annu. Rev. Pharmacol*, **54**, 119–139.

Nocjar, C., Roth, B.L. & Pehek, E.A. (2002) Localization of 5-HT(2A) receptors on dopamine cells in subnuclei of the midbrain A10 cell group. *Neuroscience*, **111**, 163–176.

de Rios, M.D., Grob, C.S. & Baker, J.R. (2002) Hallucinogens and redemption. *J. Psychoactive Drugs*, **34**, 239–248.

Shan, N.S. & Hedden, M.P. (1978) Behavioral effects and metabolic fate of N, N-dimethyltryptamine in mice preteated with beta-diethylaminoethyl-diphenylpropylacetate (SKF 525-A), improniazid and chlorpromazine. *Pharmacol. Biochem. Be.*, **8**, 351–356.

Smyth, R.L., Canton, H., Barrett, J.R. & Sandres-Bush, E. (1998) Agonist properties of N, N-dimethyltryptamine at serotonin 5-HT2A and 5-HT2c receptors. *Pharmacol. Biochem. Be.*, **61**, 323–330.

Thomas, G., Lucas, P., Capler, N.R., Tupper, K.W. & Martin, G. (2013) Ayahuasca-assisted therapy for addiction: results from a preliminary observational study in Canada. *Curr. Drug. Abuse Rev.*, **6**, 30–42.

Ting-A-Kee, R., Vargas-Perez, H., Bufalino, M.R., Bahi, A., Dreyer, J.L., Tyndale, R.F. & van der Kooy, D. (2013) Infusion of brain-derived neurotrophic factor into the ventral tegmental area switches the substrates mediating ethanol motivation. *Eur. J. Neurosci.*, **37**, 996–1003.

Vaidya, V.A., Marek, G.J., Aghajanian, G.K. & Duman, R.S. (1997) 5-HT2A receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. *J. Neurosci.*, **17**, 2785–2795.

Vaidya, V.A., Terwilliger, R.M. & Duman, R.S. (1999) Role of 5-HT2A receptors in the stress-induced down-regulation of brain-derived neurotrophic factor expression in rat hippocampus. *Neurosci. Lett.*, **262**, 1–4.

Vargas-Perez, H. & Doblin, R. (2013) The potential of psychedelics as a preventative and auxiliary therapy for drug abuse. *Curr. Drug. Abuse Rev.*, **6**, 1.

Vargas-Perez, H., Ting-A-Kee, R.A., Heinmiller, A., Sturgess, J.E. & van der Kooy, D. (2007) A test of the opponent-process theory of motivation using lesions that selectively block morphine reward. *Eur. J. Neurosci.*, **25**, 3713–3718.

Vargas-Perez, H., Ting-A-Kee, R. & van der Kooy, D. (2009a) Different neural systems mediate morphine reward and its spontaneous withdrawal aversion. *Eur. J. Neurosci.*, **29**, 2029–2034.

Vargas-Perez, H., Ting-A Kee, R., Walton, C.H., Hansen, D.M., Razavi, R., Clarke, L., Bufalino, M.R., Allison, D.W. *et al.* (2009b) Ventral tegmental area BDNF induces an opiate-dependent-like reward state in naive rats. *Science*, **324**, 1732–1734.

Vargas-Perez, H., Bahi, A., Bufalino, M.R., Ting-A-Kee, R., Maal-Baredm, G., Lam, J., Fahmy, A., Clarke, L. *et al.* (2014) BDNF signaling in the VTA links the drug-dependent state to drug withdrawal aversions. *J. Neurosci.*, **34**, 7899–7909.

Walters, K.J., Sheard, M.H. & Davis, M. (1978) Effects of N, N-Dimetthyltryptamine (DMT) and 5-Methoxy-N, N-dimethyltryptamine (5-MeODMT) on shock elicited fighting in rats. *Pharmacol. Biochem. Be.*, **9**, 87–90.

White, F.J. (1996) Synaptic regulation of mesocorticolimbic dopamine neurons. *Annu. Rev. Neurosci.*, **19**, 405–436.

World Health Organization. (2011) Global Status Report on noncommunicable diseases 2010. Available http://www.who.int/nmh/publications/ncd_report_full_en.pdf

Yuen, E.Y., Jiang, Q., Chen, P., Feng, J. & Yan, Z. (2008) Activation of 5-HT2A/C receptors counteracts 5-HT1A regulation of n-methyl-D-aspartate receptor channels in pyramidal neurons of prefrontal cortex. *J. Biol. Chem.*, **283**, 17194–17204.

Zhong, P., Yuen, E.Y. & Yan, Z. (2008) Modulation of neuronal excitability by serotonin-NDA interactions in prefrontal cortex. *Mol. Cell Neurosci.*, **38**, 290–299.