Therapeutic-like properties of a dopamine uptake inhibitor in animal models of amphetamine addiction

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Abstract

N-substituted benztroline (BZT) analogs are molecules that display high affinity for the dopamine transporter (DAT), therapeutic-like effects in animal models of cocaine abuse, and psychopharmacological characteristics consistent with those of a substitute medication for cocaine addiction. Since amphetamine (Amph) and cocaine share mechanisms of action at the DAT, we evaluated the effectiveness of a BZT analog in animal models of Amph addiction. We tested in mice and rats the effects of the BZT derivative, 3c-[bis(4-fluorophenyl)methoxy]tropane (AHN-1055), on Amph-induced conditioned place preference (CPP), locomotor activity, sensitization, self-administration and ΔFosB accumulation in the nucleus accumbens (NAc). The results showed that AHN-1055 did not produce rewarding, stimulant, or sensitized locomotor effects in mice when administered alone but it readily blocked the rewarding, stimulant, and sensitizing effects of repeated Amph exposure. Furthermore, in mice undergoing conditioning in the CPP paradigm, the BZT analog prevented the accumulation of ΔFosB protein induced in the NAc shell region by Amph treatment. Notably, treatment with AHN-1055 dose-dependently reduced Amph self-administration in rats with a steady history of voluntary Amph intake. These results provide a straightforward demonstration that a BZT derivative with binding affinity for DAT exhibits high efficacy in animal models of Amph abuse, suggesting that the novel generation of BZT analogs could have wider therapeutic applications in stimulant-spectrum disorders than those previously recognized.

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Introduction

Amphetamine (Amph) is a potent psychoactive drug that produces euphoria and elevated alertness, wakefulness, and motivation by enhancing the activity of the central and peripheral nervous systems. Amph has some medicinal applications, currently being prescribed for the treatment of attention deficit hyperactivity disorder, narcolepsy and obesity (Dopheide & Pliszka, 2009; Fleckenstein et al. 2007; Nishino, 2007). However, Amph is most often used for non-medical purposes due to its strong stimulant, rewarding and reinforcing properties. Among psychomotor stimulant drugs (a generic term that includes illicit substances such as cocaine, Amph and methamphetamine), Amph is one of the most commonly used worldwide. Recent surveys carried out in North America and Europe indicate that trends of trafficking and abuse of Amph and methamphetamine remain alarmingly high (European Monitoring Centre for Drugs and Drug Addiction, 2009; United Nations Office on Drugs and Crime, 2009). Notwithstanding these facts and trends, no specific medications are currently approved by the US Food and Drug Administration and the European Medicines Agency to treat addiction to Amph and Amph-related substances.
Amph exerts behavioural and cognitive effects by modulating the activity of monoaminergic neurotransmitters. While several studies have shown that some of the effects of Amph are partially attributable to activation of norepinephrine and serotonin pathways (Rothman & Baumann, 2006; Sofuoglu & Sewell, 2009), there is a consensus that its stimulant and euphorogenic actions are mediated primarily by the dopamine (DA) system (Drevets et al. 2001; Lott et al. 2005). The neuronal mechanisms by which Amph increases DA concentrations have been studied extensively. Once Amph has entered into the cytosol of DA neurons, both via the dopamine transporter (DAT) and through diffusion across the cell membrane, it interacts with vesicular monoamine transporters to release endogenous stores of DA (Schmitz et al. 2001; Sulzer et al. 2005). The accumulation of cytosolic DA, which is further enhanced by the inhibitory effects of Amph on monoamine oxidase, then promotes the reverse transport of DA through the DAT (Robertson et al. 2009). An important aspect to consider is that recent observations indicate that DA, cocaine, and Amph all share overlapping binding sites at the DAT (Beuming et al. 2008; Indarte et al. 2008). However, recent studies have shown that the differential behavioural effects of cocaine and other uptake inhibitors may partially derive from variations in the mode of interaction with the DAT, producing different changes in the molecular conformation of DAT protein (Chen et al. 2004; Loland et al. 2008). Thus, molecules that might interact with the DAT to impede the actions of Amph and cocaine at the DAT without producing by themselves psychomotor stimulant-like effects could serve as potential medications for stimulant abuse.

N-substituted benztropine (BZT) analogs are rationally designed, high-affinity DA uptake inhibitors with such desirable properties (Agoston et al. 1997; Katz et al. 2001). BZT derivatives bind in the vestibule at a site within the DAT overlapping with that of DA (Beuming et al. 2008), thereby blocking uptake and potentially interfering with the same binding site targeted by cocaine and Amph at the DAT. Additionally, BZT analogs show neurochemical properties consistent with that of a replacement medication for stimulant abuse. Some BZT derivatives exhibit rates of DAT occupancy slower than that of cocaine (Desai et al. 2005) and provoke slow and sustained increases in extracellular DA levels (Raje et al. 2002, 2005; Tanda et al. 2009). Moreover, several of these compounds have been shown to lack cocaine-like properties. BZT analogs do not readily evoke locomotor stimulant, rewarding and reinforcing effects (Hiranita et al. 2009; Katz et al. 2001) and have, additionally, the ability to block most cocaine-related behaviours, including reward and self-administration (Ferragud et al. 2009; Velazquez-Sanchez et al. 2009).

Given that interference with the DAT largely accounts for the psychostimulant effects of cocaine and Amph, we tested the hypothesis that BZT analogs could be effective in animal models of Amph abuse. Here, we used 3α-[bis(4-fluorophenyl)methoxy]tropane (AHN-1055), a relatively inert BZT analog with similar affinity for DAT and muscarinic M1 receptors, and with therapeutic-like properties in models of cocaine abuse (Ferragud et al. 2009; Velazquez-Sanchez et al. 2009). We used neurochemical and behavioural assays in which AHN-1055 was given alone and in combination with Amph to gain insight into the possible application of BZT derivatives as pharmacotherapies for Amph addiction.

Materials and methods

Subjects

Male Swiss OF-1 mice (n = 144, Charles River, Spain), aged 5–6 wk and weighting 22–26 g, and male Long Evans rats (n = 29, colony based at the Servei Central de Support a la Investigació Experimental, Spain) aged 8–10 wk and weighting 250–300 g, served as subjects. Rats and mice were allowed to acclimatize for at least 1 wk prior to any experimental manipulation. Mice were housed in groups of four and rats were housed individually after surgery. The housing rooms were maintained under standard conditions of temperature (21 ± 2 °C) and humidity (45–55%) and were kept on a reversed 12-h light/dark cycle (lights on 21:00 hours). Mice were given food and water ad libitum and rats were given 20 g of rodent chow per day with free access to water. All experiments were performed in accordance with current European directives regulating animal experimentation (86/609/ECC) and were approved by the Ethical Committee (Faculty of Pharmacy) of the University of Valencia.

Pharmacological treatments

AHN-1055 was synthesized as described previously (Agoston et al. 1997; Ferragud et al. 2009). Purity of the product was assessed by magnetic resonance and was found to exceed 98%. AHN-1055 was dissolved in 0.9% saline, sonicated for complete solubilization and injected at doses of 0, 3, and 10 mg/kg i.p. in mice and at doses of 0, 5 and 10 mg/kg i.p. in rats. Dosage of the BZT analog was based on previous experiments.
(Velazquez-Sanchez et al. 2009). Amph sulphate (Sigma-Aldrich, UK) was dissolved in 0.9% saline and injected at doses of 0, 0.5, and 4 mg/kg i.p. in mice. Low and high doses of Amph were deliberately chosen to examine potential up-shift/down-shift effects of the BZT analog on Amph-induced responses. In the self-administration assays Amph was available at a dose of 0.1 mg/kg per infusion in 100 μl (Cain et al. 2008).

Surgery

For the intravenous self-administration experiments, rats were anaesthetized with Avertin (2,2,2-tribromoethanol, 12.5 mg/ml, in 2.5% tertiary amyl alcohol, 2 ml/100 g of body weight), and catheters (o.d. 0.63 mm, i.d. 0.30 mm, Camcaths, UK) were implanted into the right jugular vein, exiting dorsally between the scapulae. Rats were treated post-surgically with daily injections of antibiotic (Baytril®, 10 mg/kg s.c.; Bayer, Germany) for 7 d. Catheters were flushed with heparinized saline (0.1 ml, 70 IU/ml) before and after injections.

Behavioural assays

Conditioned place preference (CPP) was carried out in chambers made of Perspex as described previously (Velazquez-Sanchez et al. 2009). The CPP procedure consisted of three phases: pre-conditioning, conditioning, and post-conditioning. During pre-conditioning, mice were habituated to the apparatus for 15 min on 2 consecutive days, the last of which (pre-conditioning session) was taken as baseline. The behaviour of the mice in the CPP apparatus was monitored using a video-tracking system (Viewpoint 2.5, France) that provided automated measures of time spent in each compartment and distance travelled, providing estimations of preference and locomotor activity, respectively. Mice that spent more than 70% of the time in one of the compartments during baseline (n = 15) were excluded from the study. Mice were assigned to nine experimental groups (n = 9–13 per group) receiving saline or AHN-1055 (3 and 10 mg/kg) as a pretreatment followed by saline or Amph (0.5 and 4 mg/kg) 60 min later. Conditioning was performed over 8 consecutive days, alternating drug sessions with control sessions in which mice received saline injections. During conditioning, the drug treatments were administered and the mice were placed individually into the drug-paired compartment for 30 min. Treatments and compartments (decorated with either circles or stripes) were counterbalanced. The post-conditioning session was performed 24 h after the last conditioning session. Mice were placed in the CPP apparatus in a drug-free state and were allowed to explore it for 15 min with the guillotine doors removed. The time spent in each of the compartments was recorded. The change induced by the treatments in the preference for one compartment or the other (pre-conditioning vs. post-conditioning tests) was estimated as the ratio between the percentage of time spent in the drug-paired compartment and the time spent in the vehicle-paired compartment (Hernandez-Rabaza et al. 2008; Velazquez-Sanchez et al. 2009). Animals were sacrificed 60 min after the test session and the brains were used for immunocytochemistry.

The sensitization and stereotypy assays were performed in Perspex boxes (53 x 28 x 15 cm). Mice were habituated to the apparatus for 30 min the day before the assays began. Mice were distributed into four experimental groups (n = 6 per group) and received saline or AHN-1055 (10 mg/kg i.p.) for 4 consecutive days as a pretreatment followed 60 min later by challenge with saline or Amph (4 mg/kg i.p.). Mice were monitored with a video-tracking system (Viewpoint 2.5, France) for 20 min. Two trained observers blind to the experimental treatments took measures of stereotyped behaviour using a rating scale (Velazquez-Sanchez et al. 2009) 10 and 20 min after the challenge with saline or Amph.

Rats (n = 6–8 per group) were trained to lever-press in operant chambers (Panlab S.L., Spain) for Amph infusions (0.1 mg/kg per infusion in 100 μl/5 s). Operant boxes were fitted with two retractable levers serving as active and inactive levers in a counterbalanced fashion. Active lever presses resulted in infusions of saline or Amph, illumination of a stimulus light for 5 s and retraction of the levers for 30 s. Inactive lever presses had no programmed consequences. Rats were trained on a fixed-ratio (FR1) schedule of reinforcement for 1-h sessions (Ferragud et al. 2009). Priming injections of Amph were never given. Pretreatments with the BZT analog were introduced only when rats had attained consistent levels of Amph self-administration. The criteria for stability were: days of training >10, number of responses in active lever >10 per session, and inter-session variation <20% in the last three sessions before the tests. When the criteria were achieved, saline or AHN-1055 (5 or 10 mg/kg i.p.) injections were administered in a counterbalanced fashion 60 min prior to Amph intake tests. To prevent carry-over effects and/or cumulative effects of the BZT analog, rats were exposed to one of the two AHN-1055 doses, counterbalanced with saline injections.
Immunocytochemistry and microscopy

Mice used in the CPP experiment were perfused under pentobarbital anaesthesia (100 mg/kg i.p.) with 0.9% saline followed by paraformaldehyde in phosphate buffer 75 min after the post-conditioning test was performed. Brains were removed, post-fixed and cut in 35-μm coronal sections on a cryostat. Sections were treated with 3% H₂O₂ to block endogenous peroxidase for 10 min and with 5% normal goat serum for 30 min. Samples were incubated with ΔFosB antibody (1:500, Santa Cruz Biotechnology, USA) overnight at room temperature and exposed to secondary antibody (goat anti-rabbit IgG 1:400, Vector Laboratories, USA) and to HRP-conjugated streptavidin (1:5000, Vector Laboratories, USA). Tissue was reacted with diaminobenzidine–H₂O₂ complex with nickel intensification (NiSO₄) to produce a nuclear black reaction. Sections were mounted onto slides, dried, dehydrated and topped with coverslips. Analysis was analogous to that described previously (Velazquez-Sanchez et al. 2009). High resolution photomicrographs of the shell and core of nucleus accumbens (NAc) were taken at 40 × with an optical microscope (Nikon Eclipse E800). Digital photographs were taken and equalized using the corpus callosum as a blank and analysed with image analysis software (Image Tool, UTHSCSA). To obtain an average density at least four sections were studied for each subject. Sections ranging from 1.10 mm to 1.35 mm rostral to bregma (Paxinos & Franklin, 2009) were included in the analysis. Two counting frames were used for each of the sections analysed, one for the core and one for the shell subregions. The position of the frame remained constant in the core and ventromedial shell of NAc using the anterior commissure as a reference, as shown in Fig. 3b. The counting frame was 0.07 mm² for all areas examined. Threshold intensity was controlled manually within a constant range to eliminate background stain. ΔFosB-positive cells were counted by an observer blind to the treatments and the results were expressed as cell density (cells/mm²) in each of the regions studied.

Statistical analysis

Parametrical data were analysed by ANOVA followed Student Newman–Keuls (NK) post-hoc tests using the sampling error from the overall ANOVA as denominator. For the analysis of non-parametric observations, we used the procedure of Conover & Iman (1981) involving rank transformations followed by ANOVA (Velazquez-Sanchez et al. 2009) followed post-hoc comparisons. Statistical significance was established at α = 0.05 per experiment.

Results

ΔFosB expression in NAc core 

AHN-1055 blocks reward, hyperlocomotion and neural adaptations induced by Amph conditioning

To test the ability of AHN-1055 to block the rewarding effects of the Amph we used a CPP procedure. ANOVA was performed with treatment as a between-subjects variable, with nine levels (nine experimental groups) and conditioning as a within-subjects variable, with two levels (pre-conditioning and post-conditioning). ANOVA indicated a significant treatment × conditioning interaction (F(8, 270) = 2.264, p = 0.0292). There were no significant differences between experimental groups in the baseline levels of preference for one compartment or the other. Post-hoc analysis revealed that Amph exposure produced significant CPP only at the high dose (p < 0.05 by NK test) whereas AHN-1055 treatment did not produce any preference or aversion at any dose, as reported previously (Velazquez-Sanchez et al. 2009). Remarkably, pretreatment with AHN-1055 dose-dependently blocked the preference induced by 4 mg/kg Amph (Fig. 1). Locomotor activity was measured during the drug conditioning sessions. ANOVA for locomotor activity counts (i.e. distance travelled) was performed with treatment as a between-subjects variable, with nine levels (nine groups of treatment) and session as a within-subjects variable, with four levels (4 d of drug administration). ANOVA showed a significant effect of the treatment variable (F(8, 90) = 32.348, p < 0.0001) and a significant treatment × session interaction (F(8, 270) = 2.945, p < 0.0001). Amph potently induced locomotor activity at the high dose (p < 0.05 by NK test), while the low dose was ineffective. Pretreatment with the high dose of AHN-1055 significantly attenuated Amph-induced hyperactivity (p < 0.05 by NK test). Such attenuation ranged from 45% to 60% across the four sessions of Amph conditioning (Fig. 2).

In order to examine the neural mechanisms underlying the ability of the BZT analog to prevent the rewarding and stimulant effects of repeated Amph treatment mice were sacrificed after the conditioning test and ΔFosB accumulation in the NAc was quantified (Fig. 3). Amph increased ΔFosB expression in NAc core (F(3, 32) = 7.302, p = 0.0007, p < 0.05 by NK test) and NAc shell (F(3, 31) = 9.439, p = 0.0001, p < 0.05 by NK test) after 4 d of treatment. There was a tendency for the BZT analog to inhibit the constitutive expression of ΔFosB in the shell region when administered alone (p < 0.10 by NK test). In parallel with the behavioural observations, pretreatment with AHN-1055 attenuated Amph-induced ΔFosB accumulation in the NAc. Such decrease was significant in
the shell region, reaching 62% (*p* < 0.05 by NK test) (Fig. 3).

**AHN-1055 prevents Amph-induced locomotor sensitization**

Amph is known for its ability to induce sensitization to its own locomotor stimulant effects (Pierce & Kalivas, 1997). To determine whether pretreatment with AHN-1055 would influence the expression of Amph sensitization, mice were exposed to daily injections of Amph and locomotor stimulation was measured. Concurrently, measures of stereotyped behavior were taken to control for the induction of motor responses typically associated with excessive dopaminergic stimulation. ANOVA was performed with two between-subjects variables. We calculated ANOVA for locomotor activity counts (i.e. distance travelled) with treatment as a between-subjects factor, with four levels (four experimental groups) and session as a repeated-measures variable, with four levels (4 d of drug administration). ANOVA yielded a significant treatment × session interaction (*F*<sub>27,180</sub> = 1.704, *p* = 0.022). Repeated exposure to AHN-1055 did not induce significant overall variations in locomotor activity across the 4 d of treatment. On the contrary, Amph injections gradually enhanced hyperactivity as treatment progressed, producing a fast-onset, significantly increased boost of locomotor behavior compared to values corresponding to acute (i.e. session 1) Amph

![Graph](image_url)
Amph-induced locomotor activity was blocked by AHN-1055 treatment throughout the experiment, including such sensitized components (Fig. 4a).

Stereotyped behaviours were measured after the administration of the drug treatments. ANOVA was performed on the ranked data with treatment as a between-subjects factor, with four levels (four experimental groups) and session as a repeated-measure variable, with four levels (4 d of drug administration). The analysis revealed a significant treatment × session interaction ($F_{9,60} = 11.074, p < 0.0001$). Amph treatment produced robust motor stereotypy that increased with repeated exposure. It should be noted that the expression of stereotypy was not incompatible with the simultaneous induction of sensitized locomotor activity, as noted previously in mice (Velazquez-Sanchez et al. 2009). Amph-induced stereotypy consisted mainly of the repetitive selection of running paths (Bonasera et al. 2008), which did not interfere with the expression of locomotor sensitization. Pretreatment with AHN-1055 induced a mild, but significant increase in stereotypy. At a dose of 10 mg/kg, AHN-1055 did not appear to attenuate significantly the motor stereotypy-induced repeated Amph treatment (Fig. 4b).

AHN-1055 dose-dependently blocks Amph self-administration

To investigate the effects of AHN-1055 on Amph intake, rats with a stable history of Amph consumption were treated with the analog 60 min prior to self-administration session. Rats achieved highly consistent levels of self-administration performance during the training phase. ANOVA for number of reinforcements during this period (i.e. reinforced active lever presses) was calculated with treatment as a between-subjects factor, with four levels (two experimental groups and two control groups) and session as a within-subjects variable, with ten levels (number of self-administration sessions). ANOVA indicated a main effect of the treatment ($F_{3,125} = 79.552, p < 0.0001$).
Following training, the treatments with the BZT analog (5 or 10 mg/kg i.p.) were introduced in a counterbalanced fashion. For data obtained during the test phase, ANOVAs were performed with treatment as a between-subjects factor, with two levels (saline or Amph) and test as a within-subjects variable, with two levels (saline or AHN-1055). Data showed that pretreatment with the BZT derivative dose-dependently blocked Amph self-administration (Fig. 5a). The reduction was significant at the high dose of AHN-1055 ($F_{1,11}=14.985$, $p=0.0026$, $p<0.05$ by NK test).

**Discussion**

The aim of the present experiments was to evaluate the therapeutic-like effects of a DAT inhibitor with low abuse liability in animal models of Amph addiction. We used a variety of clinically relevant animal models, including locomotor stimulation, sensitization, reward, and self-administration. The data showed that AHN-1055, a high-affinity DAT inhibitor, prevented the expression of key Amph-related behaviours. The novel observations presented provide a clear demonstration that the new generation of BZT derivatives may be useful leads for the development of future medications for addiction to Amph, and Amph-related drugs that share activity at the DAT.

Great efforts have been made over the last 20 yr to develop substances with therapeutic applications in stimulant abuse. Albeit psychomotor stimulants alter central norepinephrine and serotonin transmission, it is accepted that their reinforcing and euphorogenic
effects are principally mediated by the DA system. The ability of cocaine-like drugs to maintain self-administration is correlated with their binding affinity for the DAT (Ritz et al. 1987). In humans, the induction of self-reported euphoria is a function of DAT occupancy by cocaine (Volkow et al. 1996). Thus, the majority of the newly synthesized, potentially therapeutic agents have been designed to target the DAT to either ‘agonist’ or replacement medication for stimulant abuse based on DAT interference is supported by the existence of a number of DAT inhibitors whose pharmacokinetic/dynamic and behavioural profile clearly differs from that of cocaine. From a therapeutic standpoint, features that are desirable for a DAT inhibitor include slow rate of penetration into the brain, slow receptor onset and offset, prolonged psychopharmacological action and weak reinforcing efficacy. Some new BZT derivatives exhibit neurochemical and behavioural characteristics that are fitting with such desired low abuse profile (Newman & Kulkarni, 2002; Rothman et al. 2008).

BZT analogs, including AHN-1055, have been evaluated in preclinical models of cocaine addiction, with promising results (Desai et al. 2005; Ferragud et al. 2009; Hiranita et al. 2009; Velazquez-Sanchez et al. 2009, 2010), but their possible effectiveness in models of Amphetamine addiction have not been investigated until now. We first studied the effects of BZT analog treatment on the rewarding, stimulant and sensitizing effects of Amphetamine. AHN-1055 was largely devoid of strong stimulant, sensitizing and rewarding effects (Li et al. 2005; Velazquez-Sanchez et al. 2009). As predicted, AHN-1055 blocked Amphetamine CPP and significantly attenuated Amphetamine locomotor activity. When we administered Amphetamine on a daily basis, it produced a progressive increment in locomotor behaviour. Such sensitized behavioural responses were also blocked the BZT analog. The fact that AHN-1055 did not potentiate Amphetamine-induced motor stereotypy suggests that the blocking effects of the BZT analog on locomotor activity and CPP were unlikely to result from the induction of excessive psychomotor effects or potentially interfering behavioural responses. It is more reasonable to assume that AHN-1055 prevented at least some of the persistent neuroadaptations associated with Amphetamine reward and sensitization. We attempted to uncover some of the neuronal mechanisms responsible for the ability of the BZT derivative to block the behavioural effects of Amphetamine. Previous data indicated that AHN-1055 blocked cocaine-induced c-Fos protein expression in the NAc in mice undergoing conditioning. However, in the present experiments, mice were submitted to Amphetamine conditioning and were later tested for CPP. Thus we used ΔFosB accumulation, instead of c-Fos induction, as a read-out of brain activity. ΔFosB accumulates in the NAc following repeated exposure to motor stimulants (Hope et al. 1994) and such protein build-up enhances sensitivity to the rewarding effects of both natural and drug rewards, and increases motivation to seek them (Kelz et al. 1999; McClung & Nestler, 2003; Olausson et al. 2006). Interestingly, repeated AHN-1055 treatment failed to produce ΔFosB accumulation in the NAc, thus differing from other DAT blockers such as cocaine and methylphenidate, which do induce accumulation of the protein (Hope et al. 1994; Kim et al. 2009). Furthermore, the BZT analog markedly reduced ΔFosB expression after Amphetamine conditioning. Such effect was most evident in the shell region of the NAc, which is, by virtue of connections with the hypothalamus and the ventral tegmental area, a key nodal point of the limbic circuitry that subserves appetitive behaviour, viscerosomatic responses and the unconditioned effects of motivationally salient stimuli, including drugs of abuse (Kelley, 1999; Meredith et al. 2008). Our data suggest that one of the mechanisms by which AHN-1055 prevents the behavioural effects of Amphetamine is by interference with the changes of gene expression that accompany repeated drug exposure. Such changes in gene expression are probably secondary to the pharmacological interactions of AHN-1055 and Amphetamine, which probably result in reduced entry of Amphetamine into the cytosol and decreased DA efflux through reverse transport.

When examining the blocking effects of AHN-1055 on Amphetamine-induced CPP, we must consider that other factors, in addition to reward, could be implicated. Place conditioning requires the simultaneous processing of the unconditioned stimulus (i.e. the drug), the formation of a cognitive representation of the context in which conditioning takes place, the association between the drug and the contextual representation, and the long-term memory of such association (Tzschentke, 2007). AHN-1055, unlike the majority of addictive drugs, failed to induce CPP when administered alone. Similarly, there was no conditioning when the BZT analog was administered prior to Amphetamine. The data do not allow us to establish whether AHN-1055 blocked Amphetamine-induced reward, or instead interfered with some of the aforementioned learning processes. Yet, the strong association that exists between ΔFosB expression in the NAc and sensitivity to drug-induced...
and natural reward (McClung & Nestler, 2003; Olausson et al. 2006) favours the hypothesis that AHN-1055 blocked Amph reward, although further investigation is warranted.

Having shown therapeutic-like effects of AHN-1055 in models of Amph reward and psychomotor stimulation, we next probed whether the BZT analog could act as a replacement treatment in an animal model of Amph self-administration. Previous observations indicated that a range of BZT analogs, including AHN-1055, retain in some measure cocaine-like discriminative properties, substituting for cocaine only partially (Katz et al. 1999, 2001). Self-administration of AHN-1055 is less robust than self-administration of cocaine, and does not seem to generate context-induced drug-seeking after abstinence (Ferragud et al. 2009), thus suggesting that the BZT analog has low abuse liability. Further, Ferragud et al. (2009) reported that AHN-1055 dose-dependently reduced cocaine self-administration. In the present experiments, acute treatment with the BZT analog reduced Amph intake in a dose-dependent manner in rats with solid training in Amph self-administration. Thus AHN-1055 did not behave as an antagonist in this model.

In animal models of stimulant self-administration, DA receptor antagonists typically cause a shift in the dose–response curve to the right, increasing responding as a means to compensate for the reduced subjective value of the self-administered stimulant (Ahmed & Koob, 2004; Koob et al. 1987; Phillips et al. 1994). Although we did not control for intoxicating or stereotypy-inducing effects of AHN-1055 in the current experiments, we consider it unlikely that the pharmacological regimen to which the rats were subjected interfered with the task. A similar treatment with AHN-1055 did not reduce, but rather increased, lever presses for sucrose reinforcement (Ferragud et al. 2009), making it unlikely that the BZT analog caused motoric impairments hampering the operant task. By contrast, AHN-1055 significantly reduced the intake of Amph, suggesting that to some extent the BZT analog substituted for Amph, in spite of exhibiting behavioural characteristics that are clearly distinct from Amph, cocaine and other DAT inhibitors, such as nomifensine (Ferragud et al. 2009; Velazquez-Sanchez et al. 2009).

AHN-1055 is an antagonist at muscarinic M₁ receptors and therefore we cannot rule out that actions at these receptors could partially account for the behavioural effects observed in the Amph interaction assays. However, most current evidence would argue against this possibility. M₁ receptor deficiency leads to elevated dopaminergic transmission and heightened locomotor response to Amph, suggesting that tonic M₁ receptor activation opposes, rather than facilitates, DA actions (Gerber et al. 2001). Furthermore, the selective M₁ antagonist, MT-7, did not alter the motor responses to Amph (Wirtshafter, 2006). In addition, data with the DAT inhibitor, JHW007, which exhibits much weaker relative affinity for M₁ receptors, suggest that antagonistic actions at these receptors are not required to prevent cocaine-induced reward and locomotor stimulation (Velazquez-Sanchez et al. 2010). However, the anticholinergic profile of some BZT derivatives should be investigated further in relation to their potential as pharmacotherapies for stimulant addiction.

The further development and evaluation of BZT-related analogs is important and timely. The use of medications that could act as functional antagonists under conditions of high dopaminergic tone and as agonists under conditions of DA depletion would represent a major advancement in the management of stimulant addiction. Such medications could act as antagonists when DA tone is elevated during active drug-seeking and taking (e.g. following cocaine or Amph intake), thus serving as detoxifying agents. On the other hand, they could serve as agonists when DA tone is low during abstinence (i.e. hypodopaminergia associated with stimulant withdrawal) by blocking uptake and ‘normalizing’ DA transmission, thus reducing the likelihood of relapse. In animal models of cocaine addiction AHN-1055 has shown properties that are consistent with such a profile. The results of the current experiments widen the spectrum of the possible therapeutic applications of novel DAT inhibitors in stimulant abuse. We provide the first demonstration that a BZT analog such as AHN-1055 shows characteristics that are unlike Amph in models of drug-induced reward, locomotor stimulation, sensitization and gene expression, and exhibits therapeutic-like properties by blocking both Amph-related behaviours and key plasticity changes that accompany chronic stimulant exposure. These features are all much sought after in a dual agonist/antagonist medication for stimulant abuse.

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Statement of Interest
None.

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