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(54) Title: BIFEPRUNOX FOR TREATING ADDICTION

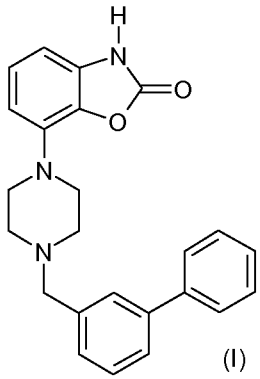
(57) Abstract: The present invention relates to bifeprunox for use in the treatment of a patient with a substance use disorder, in particular wherein the substance is alcohol or nicotine and or food addiction, particularly where the addictive food is sucrose, a mono-or a polysaccharide.

BIFEPRUNOX FOR TREATING ADDICTION

The present invention relates to the use of bifeprunox for the treatment of a patient with a substance use disorder, especially nicotine abuse and dependence and/or alcohol abuse and dependence, and/or food addiction, and a method for the treatment of a patient suffering from such disorder.

BACKGROUND OF THE INVENTION

The compound which is the subject of the present invention has the general formula (I)



(I)

and its chemical name is (7-[4-([1,1'-biphenyl]-3-ylmethyl)-1-piperazinyl]-2(3H)-benzoxazolone (INN bifeprunox).

A hydrochloric acid salt of bifeprunox is described in WO97/36893 and a monomethanesulfonate salt of bifeprunox is described in WO02/066449 (7-[4-([1,1'-biphenyl]-3-ylmethyl)-1-piperazinyl]-2(3H)-benzoxazolone monomethanesulfonate, INN bifeprunox mesylate). In addition, in WO 2005/016898 different crystalline forms of bifeprunox mesylate are described, in particular the alpha crystal form. The N-oxide of bifeprunox is described in WO 2007/023141.

Bifeprunox (earlier known as DU 127090) binds to dopamine D_2-like receptors and 5-HT_{1A} receptors; it is a partial agonist at dopamine $D_{2/3}$ receptors and also a partial agonist at serotonin 5-HT_{1A} receptors.

Bifeprunox has been suggested to be useful for treatment of several diseases in the central nervous system. For instance in WO08025780 A1 it is disclosed that "bifeprunox's affinity for both the dopamine D_2 and serotonin 5-HT_{1A} receptors makes it useful for the treatment of schizophrenia and other psychotic disorders. For example, bifeprunox can be of value for the treatment of affections or diseases of the CNS (Central Nervous System) caused by disturbances in either the dopamine or

serotonergic systems, such as Parkinson's disease, aggression, anxiety disorders, autism, vertigo, depression, bipolar disorder, disturbances of cognition or memory, and further for example, schizophrenia and other psychotic disorders".

Substance use disorder is the use or misuse of, dependence on and/or addiction to a number of (psychoactive) substances as described in the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition - Revised Text (DSM-IV TR®). DSM-IV TR® also lists a wide variety of illegal psychoactive substances and drugs, eg stimulants (cocaine, amphetamine and MDMA ["ecstasy"]), sedatives (benzodiazepines, barbiturates, opioids and cannabis) and hallucinogens (LSD, phencyclidine, ketamine and "magic mushrooms"), and legal substances of abuse, *eg* alcohol and nicotine, that are causal in substance use disorder. Harmful effects of substance use disorder include drug effects (intoxication, dependence/withdrawal and accidental overdose), social problems (legal including crime to support habits, financial, occupational and family dysfunction), route of administration complications (needle sharing and non-sterile injection techniques contaminants like talc) resulting in infections such as tetanus, abscesses, HIV etc, and miscellaneous non-infectious medical disorders including psychosis, depression, neuropathy and brain damage.

Smoking is the most important preventable cause of disease, disability and death in the developed world. Diseases linked to smoking include many types of cancer (lung, esophageal, laryngeal, pancreatic, and those of the oral cavity), cardiovascular diseases, asthma, chronic obstructive pulmonary disease (COPD), emphysema and other pulmonary diseases, low bone density and osteoporosis in women, pregnancy complications and reduced male and female fertility. Although health awareness campaigns have led to a reduction in tobacco use in Western industrialised countries, smoking is an increasing problem in most developing countries.

Alcohol misuse and abuse is similarly a major cause of disease, disability and death through alcoholic liver disease (ALD), cardiovascular diseases (cardiomyopathy, coronary heart disease [CHD], hypertension, arrythmias and stroke), and various forms of cancer (mouth, pharynx, larynx, esophagus, stomach, colon, rectum and breast) as well as the negative social consequences (impaired productivity, accidental injury, family disruption, domestic and non-domestic violence). Although alcohol consumption varies across cultures and ethnic groups,

excessive alcohol consumption and alcohol addiction are a major cause for concern in many countries, and in addition, binge drinking amongst young people is a growing problem that can lead to alcohol addiction in later.

It is a commonly accepted truth that heavy smokers also tend to consume alcohol excessively and *vice versa*. The link between these 2 substance use disorders has been noted in several epidemiological studies and may be due to familial, genetic and/or social factors. What is evident is that the combination of smoking and alcohol misuse negatively impacts on health and wellbeing.

It is increasingly recognised that the consumption of highly palatable foods is both rewarding and reinforcing and this has led to the hypothesis that people can become addicted to specific foods in the same way that they become addicted to substances of abuse (Corwin & Grigson, 2009, J. Nutrition, 139: 617-619; Ifland et al., 2009, Med. Hypotheses, 72: 518-526; Davis & Carter, 2009, Appetite, 53: 1-8). Like any other addiction, food addiction has adverse effects on health and well-being through over-consumption, obesity and related metabolic disorders (Corwin & Grigson, 2009; Ifland et al., 2009; Davis & Carter, 2009). Food addiction is also believed to be causal in bingeing and binge eating disorder (Davis & Carter, 2009). Bingeing refers to the consumption of a large amount of food in a discrete period of time during which a loss of control is experienced. Mostly, high calorie food, which is perceived as "forbidden food", is eaten during a binge. Before the binge, subjects experience intense feelings of craving (an almost irresistible urge to eat) and after the binge they feel guilty, disgusted with themselves and depressed (see Jansen, 1998, Behav. Res. Ther., 36: 257-72).

It has now been found that bifeprunox can be used in the treatment of a patient with a substance use disorder and/or food addiction. The ability of bifeprunox to treat substance use disorders, especially nicotine or alcohol abuse and/or dependence, or food addiction has not previously been explored. According to the present invention, it has now been demonstrated in rodent models predictive of clinical effect that bifeprunox will be efficacious in the treatment of substance use disorders, in particular nicotine abuse and dependence, alcohol abuse and dependence, or the combination of nicotine and alcohol abuse and dependence, and in addition, food addiction. These beneficial therapeutic effects are novel, surprising and unexpected because the reference comparator drug, aripiprazole, which shares the same pharmacological

profile as bifeprunox, was found to be inactive in animal models of nicotine or alcohol abuse and dependence.

Aripiprazole was selected to be the reference comparator in these Examples nicotine or alcohol abuse and dependence because bifeprunox and aripiprazole are both dopamine $D_{2\text{-like}}$ receptor partial agonists + 5-HT_{1A} partial agonists. Aripiprazole's lack of efficacy in rodent models of alcohol abuse and dependence, as reported in this invention, is consistent with its inability to inhibit alcohol intake in alcohol-preferring (AA) rats when given acutely, or repeatedly at doses that do not impair water intake (Ingman et al., 2006, Alc. Alcoholism, 41; 391-398). The predictive validity of these findings obtained in rodents for the clinical outcome was demonstrated by the failure of aripiprazole to promote abstinence in alcohol-dependent subjects determined in a randomised, double-blind, placebo-controlled, multi-centre clinical trial (Anton et al., 2008, J Clin. Psychopharmacology, 28: 5-12).

In support of the novel and unexpected nature of the invention, bifeprunox and aripiprazole were both active in the conditioned avoidance (CAR) model of antipsychotic efficacy, but only bifeprunox was consistently active in animal models predictive of efficacy for the treatment of nicotine abuse and dependence or alcohol abuse and dependence. In addition, bifeprunox has also been shown to be efficacious in animal models predictive of efficacy in the treatment of food addiction and bingeing.

SUMMARY OF THE INVENTION

The invention relates to bifeprunox for use in the treatment of a patient with a substance use disorder and/or food addiction, to the use of bifeprunox for the preparation of a medicament for the treatment of a patient with a substance use disorder and/or food addiction, and to a method of treatment of a patient suffering from a substance use disorder and/or food addiction, comprising administering a therapeutically effective amount of bifeprunox to said patient.

DETAILED DESCRIPTION OF THE INVENTION

Bifeprunox and aripiprazole are both dopamine D_2-like receptor partial agonists, 5-HT_{1A} receptor partial agonists, and are 3rd generation, atypical antipsychotic drugs.

It has now been surprisingly demonstrated that bifeprunox is highly effective in rodent models predictive of clinical efficacy in the treatment of substance use disorders, especially nicotine or alcohol abuse and/or dependence. These models have explored various aspects of nicotine and alcohol abuse and dependence including the ability to reduce drug intake, decreasing the reinforcing effects of these drugs, preventing the expression of nicotine tolerance, and reducing craving (psychological dependence). When bifeprunox was given acutely to rats that had been acclimatised to consume an 8% alcohol solution, it dose-dependently and significantly reduced the *ad libitum* (non-operant) 24h consumption of alcohol. In a model to evaluate the effect of bifeprunox on the motivation to consume alcohol, rats were trained in an operant paradigm to lever-press to consume a 6% alcohol solution on an FR6 schedule, *i.e.* 6 lever-presses deliver a reward of a small volume of the alcohol solution. In this paradigm, acute administration of bifeprunox dose-dependently and significantly reduced operant responding for alcohol "rewards". This attenuation was of very long duration being evident at 1h, 24h, and 48h after administration of bifeprunox. In rats trained to self-administer nicotine solutions intravenously in an operant paradigm (lever-pressing to obtain nicotine infusions), bifeprunox dose-dependently inhibited nicotine self-administration. The action of bifeprunox to reduce the motivation to self-administer nicotine was observed across a wide range of nicotine doses. Nicotine abuse and dependence are partly mediated by the rapid development of sensitization to its agonist action at cholinergic nicotinic receptors. This effect can be modelled in rats by an increase in the action of nicotine to cause locomotor activity when given intermittently over a period of days. In this model, acute administration of bifeprunox dose-dependently and significantly reduced the expression of the supersensitive locomotor response of rats to nicotine. Psychological dependence (craving) for nicotine (and other substances of abuse) can be triggered by contingent cues that are associated with taking the particular legal or illegal substance. Rats were trained in an operant paradigm to self-administer nicotine in conjunction with the presentation of a visual signal (contingent cue). After a period when nicotine is withheld, lever-pressing is non-rewarded and extinguishes (extinction). However, operant responding can be reinstated by presentation of the visual cue that the rat associates with the nicotine reward, but without giving the rat access to nicotine itself. This model, therefore, measures the effect of bifeprunox on the desire to take nicotine (craving or psychological dependence) rather than its effect on taking the drug itself. Acute

administration of bifeprunox dose-dependently inhibited the cue-induced relapse to nicotine seeking behaviour demonstrating that it prevents nicotine craving and this effect was observed across a range of doses of nicotine.

Food addiction shares many clinical similarities to substance use disorder. Bifeprunox has also been shown to reduce the motivation of rats to consume large numbers of sucrose pellets in an operant task where lever-pressing was reinforced by the delivery of sucrose pellets. Bifeprunox also reduced the cue-induced reinstatement of sucrose seeking behaviour (craving).

In contrast to the reported efficacy of bifeprunox in models of nicotine or alcohol abuse and dependence, aripiprazole was either inactive or transiently efficacious in these rodent models that are predictive efficacy in the treatment of alcohol or nicotine abuse and dependence. Thus, acute administration of aripiprazole to rats had no significant effect on the *ad libitum* (non-operant) 24h consumption of alcohol. In the second model where rats were trained to lever-press for access to a 6% alcohol solution on an FR6 schedule, acute administration of aripiprazole reduced operant responding for alcohol "rewards". However, its effect was transient being observed at 1h, but not at longer post-treatment intervals. In rodent models predictive of efficacy in the treatment of nicotine abuse and dependence, aripiprazole failed to reduce the expression of nicotine-induced sensitization in rats.

A comparison of the receptor binding profile of aripiprazole, which is a marketed antipsychotic, its pharmaceutical use, formulation and manufacturing is described for instance in US 5006528 and US 7115587 and also in the Examples section of this document.

Accordingly, the present invention relates to a new pharmaceutical use of bifeprunox (with the general structural formula I), including pharmaceutically acceptable salts thereof. In particular, the use of bifeprunox in the treatment of a patient with a substance use disorder and/or food addiction or bingeing.

In an embodiment, the substance in the substance use disorder is a psychoactive substance selected from alcohol, tobacco (nicotine), stimulants, sedatives, euphoriants, entactogens and hallucinogens. Particularly, the substance is a psychoactive substance, being alcohol. Further preferred is said use of bifeprunox, where the psychoactive substance is tobacco (nicotine).

In a further embodiment, in case bifeprunox is used to treat food addiction or bingeing, the food is highly palatable and calorie dense food and in particular contains sucrose or another mono- or polysaccharide as a nutrient.

For a use as described herein related to treatment of substance use disorder and/or food addiction or bingeing, the dose, preferably a daily dose, of bifeprunox calculated as the free base, is suitably at least 0.5 mg. In further embodiment, the dose, of bifeprunox for said treatment is 0.5-12 mg, in particular 2-8 mg.

In a further embodiment specific aspects of a substance use disorder can be treated using bifeprunox according to the invention, said aspects being substance abuse or substance dependence. Further, in case bifeprunox is used to treat food addiction, the aspects of food addiction are selected from food bingeing, food dependence or food craving.

In one embodiment, the pharmaceutically acceptable salt is a mesylate salt, preferably in the form of a crystalline mesylate salt of bifeprunox. The bifeprunox mesylate may be chosen from the α, γ, or δ crystalline polymorphic forms, and mixtures thereof. In a particular embodiment, the compound is in the crystal form α (alpha) of bifeprunox mesylate salt. The mesylate salt and its preparation are described in WO02/066449, whereas the α crystal form of the salt and its preparation and its physicochemical parameters are described in WO 2005/016898.

As used herein, the term "bifeprunox" refers to the active compound 7-[4-([1,1'-biphenyl]-3-ylmethyl)-1-piperazinyl]-2(3H)-benzoxazolone, its N-oxide and pharmaceutically acceptable salts, solvates and hydrates thereof and solvates and hydrates of the salts. When a salt or the N-oxide is used as the bifeprunox compound, the amount in milligrams is the same amount as the amount the person skilled in the art would select for the bifeprunox compound without the salt counterion or the oxide, respectively. In addition, pharmaceutically acceptable salts of bifeprunox or its N-oxide may be obtained using standard procedures well known in the art, for example, by mixing a bifeprunox compound of the present invention with a suitable acid, for instance an inorganic acid or an organic acid.

In one embodiment, the compound used in the present invention is in a purified form. The term "purified form" is intended to indicate that the compound is essentially free of other compounds or other forms, such as polymorphic forms of the compound, as the case may be.

In one embodiment the patient to be treated has been diagnosed with the substance use disorder for which the patient is being treated.

In a further embodiment, the patient is treated for a long term, in particular three months, six months, a year, or longer.

In the present context the terms "treatment" and "treating" means the management and care of a patient for the purpose of combating a disease, disorder or condition, such as substance use disorder, or reducing the severity of a disease, disorder or condition. The term is intended to include the full spectrum of treatments for a given disease, disorder or condition as described herein from which the patient is suffering, such as administration of the active compound to alleviate or relieve the symptoms or complications, to delay the progression of the disease, disorder or condition, to alleviate or relieve the symptoms and complications, and/or to cure or eliminate the disease, disorder or condition as well as to prevent the disease, disorder or condition, wherein prevention is to be understood as the management and care of a patient for the purpose of combating the disease, condition, or disorder and includes the administration of the active compound to prevent the onset of the symptoms or complications. Nonetheless, prophylactic (preventive) and therapeutic (curative) treatments are two separate aspects of the invention. The patient to be treated is preferably a mammal, in particular a human being.

As used herein, the phrase “effective amount” when applied to a compound of the invention, is intended to denote an amount sufficient to cause an intended biological effect. The phrase “therapeutically effective amount” when applied to a compound of the invention is intended to denote an amount of the compound that is sufficient to ameliorate, palliate, stabilize, reverse, slow or delay the progression of a disease, disorder or condition state, or of a symptom of the disease, disorder or condition.

In an embodiment of this invention, a daily dose of bifeprunox is administered as a once-a-day pharmaceutical formulation. In another embodiment, the daily dose is administered in two portions, for instance in a morning and an evening pharmaceutical preparation.

Mixed with pharmaceutically suitable auxiliaries, *e.g.* as described in the standard reference “Remington, The Science and Practice of Pharmacy” (21st edition, Lippincott Williams & Wilkins, 2005, see especially Part 5: Pharmaceutical Manufacturing) bifeprunox or a salt thereof may be compressed into solid dosage

units, such as pills or tablets, or be processed into capsules or suppositories. By means of pharmaceutically suitable liquids the compounds can also be applied in the form of a solution, suspension or emulsion.

In the preparation of the compositions of the present disclosure, the active ingredients, i.e., a bifeprunox compound, may be mixed with solid, powdered ingredients, such as lactose, saccharose, sorbitol, mannitol, starch, amylopectin, cellulose derivatives, gelatin, or another suitable ingredient, as well as with disintegrating agents and lubricating agents such as magnesium stearate, calcium stearate, sodium stearyl fumarate and polyethylene glycol waxes. The mixture may then be processed into granules, pressed into tablets, and/or any other known pharmaceutical form such as suppositories and/or suspensions.

Soft gelatin capsules may further be prepared containing a composition comprising a mixture of the active ingredients of the invention, vegetable oil, fat, or other suitable vehicle for soft gelatin capsules. Hard gelatin capsules may contain granules of the active ingredients. Hard gelatin capsules may also contain the active ingredients in combination with solid powdered ingredients such as lactose, saccharose, sorbitol, mannitol, potato starch, corn starch, amylopectin, cellulose derivatives or gelatin.

In addition, compositions of the present disclosure can comprise at least one pharmaceutical excipient. Non-limiting examples of suitable excipients include suspending agents (for example, gums, xanthans, cellulosics and sugars), humectants (for example, sorbitol), solubilizers (for example, ethanol, water, PEG and propylene glycol), surfactants (for example, sodium lauryl sulfate, Spans, Tweens, and cetyl pyridine), preservatives, antioxidants (for example, parabens, and vitamins E and C), anti-caking agents, coating agents, chelating agents (for example, EDTA), stabilizers, antimicrobial agents, antifungal or antibacterial agents (for example, parabens, chlorobutanol, phenol, sorbic acid), isotonic agents (for example, sugar, sodium chloride), thickening agents (for example, methyl cellulose), flavoring agents (for example, chocolate, thalmantin, aspartame, root beer or watermelon or other flavorings stable at pH 7 to 9), anti-foaming agents (*e.g.*, simethicone, Mylicon®), disintegrants, flow aids, lubricants, adjuvants, colorants, diluents, moistening agents, preservatives, carriers, binders (for example, hydroxypropylmethylcellulose, polyvinyl pyrilodone, other cellulosic materials and starch), diluents (for example, lactose and other sugars, starch, dicalcium phosphate and cellulosic materials),

disintegrating agents (for example, starch polymers and cellulosic materials), glidants and water insoluble or water soluble lubricants or lubricating agents.

By "pharmaceutically acceptable" it is meant that the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

In an embodiment of the invention, a pharmaceutical pack or kit is provided comprising one or more containers filled with one or more pharmaceutical compositions of the invention. Associated with such container(s) can be various written materials such as instructions for use, or a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals products, which notice reflects approval by the agency of manufacture, use, or sale for human or veterinary administration.

LEGENDS TO THE FIGURES

Figure 1. Inhibition by acute administration of bifeprunox of *ad libitum* (non-operant) 24h consumption of alcohol by male Sprague-Dawley rats – Attenuation of alcohol consumption (substance use). Data are mean ± SEM (n = 11 rats/group). Lowest effective dose (LED) = 0.01 mg/kg, p.o. Vehicle (1% methylcellulose solution) did not significantly alter the intake of an 8% ethanol solution. Significantly different from appropriate untreated control * $p < 0.05$.

Figure 2. Lack of effect of acute administration of aripiprazole (0.03 - 6.0 mg/kg, p.o.) on the *ad libitum* (non-operant) 24h consumption of alcohol by male Sprague-Dawley rats – Absence of effect on alcohol consumption (substance use). Data are mean ± SEM (n = 7-10 rats/group). The consumption of the 8% ethanol solution was reduced by oral administration of the drug vehicle (1% methylcellulose in water) on a single occasion. Significantly different from appropriate untreated control * $p < 0.05$.

Figure 3. Inhibition by acute administration of bifeprunox (0.003 - 0.03 mg/kg, p.o.) of operant (lever-press) responding for access to alcohol consumption by Sprague-Dawley rats - Attenuation of the reinforcing effects of alcohol. Rats were maintained on an operant FR6 responding for the consumption of a 6% alcohol

solution. Data are mean ± SEM (n = 12 rats/group). Lowest effective dose (LED) = 0.01 mg/kg, p.o. * $p < 0.05$ significantly different from appropriate baseline consumption at 1.0h, 24h and 48h.

Figure 4. Effect of acute administration of aripiprazole (0.03 or 0.1 mg/kg, p.o.) on operant (lever-press) responding for access to alcohol consumption by Sprague-Dawley rats - Attenuation of the reinforcing effects of alcohol. Rats were maintained on an operant FR6 responding for the consumption of a 6% alcohol solution. Data are mean ± SEM (n = 12 rats/group). Lowest effective dose (LED) = 0.1 mg/kg, p.o. * $p < 0.05$ significantly different from appropriate baseline consumption only at 1.0h.

Figure 5. Effect of acute administration of bifeprunox (0.004 - 0.25 mg/kg, s.c.) on the number of nicotine infusions obtained, and on the numbers of "active" and "inactive" lever-presses - Attenuation of nicotine consumption (substance use). Rats were maintained on an FR2 schedule for intravenous nicotine infusions. The figure shows the effects of bifeprunox on the number of nicotine infusions obtained (upper panel), and on the numbers of "active" and "inactive" lever-presses (middle and lower panels, respectively). Data are mean ± SEM (n = 10 rats/group). Lowest effective dose (LED) to inhibit nicotine self-administration = 0.064 mg/kg, s.c. Bifeprunox did not significantly inhibit pressing on the "inactive", non-rewarded lever. Significantly different from appropriate vehicle control * $p < 0.05$, ** $p < 0.01$ (Newman-Keuls test).

Figure 6. Effects of acute administration of bifeprunox (0.064 mg/kg s.c.) or vehicle on self-administration of various doses of nicotine (0.005 – 0.09 mg/0.1ml/infusion) - Attenuation of nicotine consumption (substance use). The figure shows the effects of bifeprunox (0.064 mg/kg s.c.) on the number of infusions of various doses of nicotine (upper panel), and on the numbers of "active" and "inactive" lever-presses (middle and lower panels, respectively). Data are mean ± SEM. Bifeprunox did not significantly inhibit pressing on the "inactive", non-rewarded lever. Significantly different from appropriate vehicle control * $p < 0.05$.

Figure 7. Treatment protocol for experiments to investigate the effect of bifeprunox or aripiprazole on nicotine-induced sensitization in male, Wistar rats.

Figure 8. Inhibition by acute administration of bifeprunox (0.03-0.1 mg/kg, p.o.) of the expression of nicotine-induced sensitization in male, Wistar rats - Attenuation of nicotine addiction (substance dependence). Data are mean ± SEM (n = 8 rats/group). Lowest effective dose (LED) = 0.1 mg/kg, p.o. Significantly different from the respective group indicated by the bar ** $p < 0.05$.

Figure 9. Lack of effect of acute administration of aripiprazole (0.3-3.0 mg/kg, p.o.) on the expression of nicotine-induced sensitization in male, Wistar rats – Lack of effect on nicotine addiction (substance dependence). Lack of effect of acute administration of aripiprazole on the expression of nicotine-induced sensitization in male, Wistar rats. Data are mean ± SEM (n = 8 rats/group).

Figure 10. Prevention by acute administration of bifeprunox (0.004-0.25 mg/kg, p.o.) of the cue-induced reinstatement of nicotine seeking in male Sprague-Dawley rats - Attenuation of the nicotine craving (substance craving or psychological dependence). For comparison, the number of cues earned after reintroduction of vehicle-associated stimuli are also reported. Results are the mean ± S.E.M (n = 8). Lowest effective dose (LED) = 0.016 mg/kg s.c. Data were analyzed by one-way ANOVA for repeated measurements (with session as main factor) followed by Newman-Keuls *post-hoc* comparison. [a] Significantly different ($p < 0.05$) from vehicle-associated cues; [b] Significantly different ($p < 0.05$) from the number of nicotine-associated cues earned after vehicle treatment.

Figure 11. Effect of acute administration of bifeprunox on the number of sucrose pellets earned, and on the numbers of "active" and "inactive" lever-presses - Attenuation of the reinforcing effects of sucrose. The figure shows the effects of bifeprunox (0.004 - 0.25 mg/kg s.c.) on the number of sucrose pellets earned (upper panel), and on the numbers of "active" and "inactive" lever-presses (middle and lower panels, respectively). Data are mean ± SEM. Bifeprunox did not significantly inhibit pressing on the "inactive", non-rewarded lever. Significantly different from appropriate vehicle control * $p < 0.05$; ** $p < 0.01$.

Figure 12. Prevention by acute administration of bifeprunox of the cue-induced reinstatement of sucrose seeking (sucrose craving) in male Sprague-Dawley rats - Attenuation of the sucrose craving (substance craving or psychological dependence) For comparison, the number of cues earned after reintroduction of the sucrose pellet- and no reward-associated stimuli are reported. Results are the mean ± S.E.M (n = 7-8). Lowest effective dose (LED) = 0.016 mg/kg s.c. Data were analyzed by two-way ANOVA for repeated measurements (with treatment and session as main factors) followed by Tukey's test comparison. [a] Significantly different ($p < 0.05$) from no sucrose-associated cue; [b] Significantly different ($p < 0.05$) from number of sucrose associated cues earned after vehicle-treatment.

The invention will be illustrated in the following non-limiting examples.

EXAMPLES

A. *Ad libitum* (non-operant) 24h consumption of alcohol

Introduction

Rats can be trained to consume to alcohol solutions and after a period of some exposure to alcohol access they will regularly consume alcohol as part of their daily fluid intake. When given free access to choose between an alcohol solution or water, they will consistently prefer to consume ~30% of their fluid intake each day from an 8% alcohol solution (~2.0 g/kg). This model can be used to determine whether drugs attenuate or enhance the preference that the rats display for consuming alcohol. If a drug decreases alcohol intake in this model, it predicts that it will reduce alcohol intake in man, and consequently, such a compound is likely to be beneficial in treating alcohol abuse and dependence.

Methods

Subjects: Male Sprague-Dawley rats were supplied by Harlan (the Netherlands). Bodyweight ranged between 150 and 174 grams at delivery and were approximatly 6 weeks of age. The animals were single-housed in Makrolon Type III cages on sawdust bedding with free access to food and separate bottles containing tap water or an 8% (v/v) solution of ethanol in water. After animals reached an average

bodyweight of 300 grams, animals were food-restricted to attain 85% of free-feeding weight in grams. The cages and room were cleaned at regular intervals each week. The room was illuminated by fluorescent lights timed to give a 12 h light-dark cycle (on 06.00h, off 18.00h), the temperature range was 20°C to 22°C and the relative humidity range was 40% to 50%. The rats were allowed to establish a consistent preference for alcohol intake for at least 4 months prior to the effects of drugs and drug candidates being investigated. At the start of treatment with bifeprunox and aripiprazole, the animals (n = 22) weighted 386 +/- 3.6 grams (mean +/- SEM).

Experimental procedure: Animals were offered two identical water bottles on the front of the cage (one on the left side and one on the right) and allowed to consume water. After 5 days, animals were allowed to consume increasing alcohol percentages by offering one water-bottle and one alcohol-bottle on alternating days (each alcohol day was alternated with a water day); the alcohol percentage was increased from 2% to 8% in steps of 1%. At 8% alcohol, the intervening water days (where animals were only allowed to consume water) were removed from the protocol. Thus, from that point onwards, animals could make a free choice each day between either water or 8% alcohol. Alcohol solutions were refreshed each week. The position of the alcohol bottle was changed during each new alcohol presentation; this was done to avoid positional bias. After animals showed stable baseline alcohol consumption, the effects of compounds were investigated. In this study, animals were allowed to consume alcohol for a minimum of 4 months. Each day at the same time-point (with a deviation ±1 hour), the two bottles were removed from the cages at weighed. After weighing, the bottles were replaced on the cages. Bodyweights of the animals was assessed on a weekly basis and used for the below-mentioned calculations.

The intake in g/kg:

$$\frac{\text{mL consumed} \times (\text{alcohol percentage used}/100) \times 0.8}{\text{bodyweight in kilogram}}$$

Drugs: The tests substances, bifeprunox and aripiprazole, were suspended in 1% methylcellulose. Ethanol was obtained from Duchem (the Netherlands) and was provided as increasing alcohol percentages at first and finally as an 8% (v/v) solution made up in distilled water

Drug treatments: Bifeprunox (0.003, 0.01 or 0.03 mg/kg p.o.), aripiprazole (0.03, 0.1, 0.3, 1, 3 and 6 mg/kg p.o.), or vehicle (1% methylcellulose) was administered at a volume of 2 ml/kg once at t = -60 min before the initiation of alcohol intake measurements on the test day. Each animal received only 1 treatment per week. During each treatment cycle, 50% of the group received a dose of either bifeprunox or aripiprazole whilst 50% of the group received an injection with the vehicle. Only animals that showed stable alcohol intake between treatment cycles were used for further testing.

Statistical Analysis: The intake of alcohol in g/kg was used for statistical calculations. The intakes in g/kg from three days before treatment were used to calculate a baseline intake value. Subsequently, the intake in g/kg on the test-day was compared to the baseline intake by means of a paired t-test. Also, the intake in g/kg at the test day between vehicle-treated and compound-treated animals was compared by means of a one-way ANOVA. Statistical analysis on these data was done by SPSS 15.0 and a p-value of less than 0.05 was taken as statistically significant.

Results

Bifeprunox

As shown in Figure 1, when rats were given a free choice to consume either water or an 8% solution of ethanol, the animals consumed ~1.5 g/kg of the alcohol solution over a given 24h period. The consumption of the 8% ethanol solution was not altered by oral administration of the drug vehicle (1% methylcellulose in water). Pretreatment with bifeprunox (0.003, 0.01 or 0.03 mg/kg p.o.) 60 min before the initiation of alcohol intake measurements on the test day produced a dose-dependent reduction of 24h alcohol consumption (Figure 1). It was found that all doses of bifeprunox above 0.01 mg/kg reduced the intake of alcohol (LED [lowest effective dose] = 0.01 mg/kg).

Aripiprazole

As shown in Figure 2, acute administration of aripiprazole (0.03, 0.1, 0.3, 1.0, 3.0 or 6.0 mg/kg p.o.) 60 min before the initiation of alcohol intake measurements on the test day did not significantly reduce the 24h intake of an 8% solution of ethanol. In this experiment, a significant reduction in 8% ethanol solution consumption was observed

on a single day after administration of drug vehicle (1% methylcellulose in water) (Figure 2); however, this result appears to be a random event because it was not replicated on any of the other test days when vehicle was administered (Figures 1 and 2).

Conclusions

The *ad libitum* consumption of the 8% ethanol solution by male, Sprague-Dawley rats was attenuated by acute administration of bifeprunox; LED = 0.01 mg/kg p.o. In contrast, aripiprazole did not significantly reduce the 24h *ad libitum* intake of an 8% ethanol solution, even when given at much higher doses, ie ≤ 6.0 mg/kg p.o.

B. Operant (lever-press) responding 1h consumption of alcohol

Introduction

Rats can be trained to work to obtain "rewards" of alcohol in a 2-lever operant paradigm. In the paradigm, the rat learns that pressing on one of two levers results in the delivery of a small volume of 6% alcohol solution ("active" lever), whilst pressing on the other lever ("inactive" lever) is not linked to the delivery of alcohol rewards. In this 2-choice paradigm, the rat has to lever press 6 times (FR6) on the "active" lever to obtain each single alcohol reward. After a period of training, the rat learns that pressing on the active lever will deliver alcohol rewards and because the rat values these rewards, it is prepared to work (lever press) to receive this reward. Each rat is also able to titrate its intake of alcohol according to its individual preference. This model can be used to determine whether drugs or drug candidates attenuate or enhance the consumption of alcohol in animals which are prepared to work to obtain alcohol rewards. If a drug decreases alcohol intake in this model, it predicts that it will reduce alcohol intake in man, and consequently, such a compound is likely to be beneficial in treating alcohol abuse and dependence.

Methods

Subjects: Male Sprague-Dawley rats were supplied by Harlan (the Netherlands). Bodyweight of the groups was around 341 +/- 6.3 grams (mean +/- sem) at the start of the experiment. Upon arrival, the animals were allowed to habituate to their surroundings for one week before experimentation commenced. The animals were

housed in pairs in type 3 Macrolon® cages on sawdust bedding in a temperature (19-22° C) and humidity (40-70%) controlled animal room on a reversed day-night cycle (lights off at 6.00 am) with food and tap water available *ad libitum*. The cages were cleaned at regular intervals each week.

Experimental procedure: After habituation, all food was removed from the cage and animals were allowed to consume a Lieber DeCarli Liquid Diet ® containing a base mix and maltodextrin (Special Diets Services). After 6 days, the mix was adjusted to contain 5% ethanol (and caloric value was adjusted). During the liquid diet, water remained available *ad libitum*. After 10 days, the liquid diet was replaced by standard lab chow and each animal received approximately 15 grams per day to maintain stable bodyweight at 85% of their free-food-weight.

During the liquid diet phase, animals were trained to lever press for food pellets (Test Diet Mlab Rodent tablet 45 mg) on an increasing fixed ratio (from FR1 to FR6) schedule of reinforcement during daily (5 days per week) sessions. Training was done in standardized Skinner boxes with a grid floor, 2 levers (one on the right side and one on the left side) at a height of 6 cm above the floor, with a cue light located above the lever, a house light, and a receptacle located between the two levers (Med Associates Inc, USA). Each training session ended after 30 minutes or when 60 food pellets were obtained. Sixty minutes after each session, the liquid diet was provided to the animals. After reaching the criteria (60 food pellets within 30 minutes), animals were allowed to lever-press for 6% ethanol v/v on a FR6 schedule of reinforcement (0.2 ml per infusion over 6.1 seconds) during daily (5 out of 7 days) 1 hour sessions. Each session started by the illumination of the house light and the insertion of both levers. After reaching an FR6 on the "active" lever, ethanol was infused into the receptacle, the levers were retracted from the cage and the cue light in the receptacle was illuminated, the cue light above the active lever was turned off. Lever-presses on the inactive lever were recorded, but without any consequences. After a minimum of 10 sessions of responding, animals were subjected to the first treatment. Between each treatment, animals are allowed to self-administer for ethanol for a minimum of 3 sessions. This was done to allow self-administration behaviour to stabilise (less than 10% deviation of 3 consecutive sessions) and allow a wash-out of possible compound effects.

Each session results in the number of "active" lever presses, the number of "inactive" lever-presses and the number of alcohol reinforcers. The number of reinforcers is used in statistical calculations and/or graphs. All data are presented as mean +/- SEM.

Drugs: The tests substances, bifeprunox and aripiprazole, were suspended in 1% methylcellulose. Ethanol was obtained from Duchem (the Netherlands) and was provided as an 6% (v/v) solution made up in distilled water

Drug treatments: Bifeprunox (0.003, 0.01 or 0.03 mg/kg p.o.), aripiprazole (0.03 or 0.1 mg/kg p.o.), or vehicle (1% methylcellulose) was administered at a volume of 2 ml/kg once at t = -60 min before the initiation of alcohol intake measurements on the test day. Animals were treated with each dose in a randomised order and each administration was separated by at least 3 normal sessions. During these 3 sessions, animals had to display stable baseline intake behaviour (identical to the intake before treatment) before another treatment started.

Statistical Analysis:

The number of reinforcers was used in statistical calculations. The number of reinforcers from the sessions between each treatment was used to calculate a baseline no. of reinforcers (average intake based on 3 sessions). The baseline value between each treatment was compared with a one-way ANOVA to verify that responding did not statistically increase or decrease between treatments. The effect of each treatment was analyzed by means of a paired samples t-test comparing the baseline no. of reinforcers with the no. of reinforcers after treatment. Statistical analysis on these data was done by SPSS 15.0 and a p-value of less than 0.05 was taken as statistically significant.

Results

Bifeprunox

As shown in Figure 3, rats trained to lever-press on an FR6 schedule to receive rewards of a 6% ethanol solution obtained between 9 - 11 rewards under baseline conditions. The number of ethanol rewards was not altered by oral administration of the drug vehicle (1% methylcellulose in water). Pretreatment with bifeprunox (0.003, 0.01 or 0.03 mg/kg p.o.) 60 min before the start of the experimental session significantly decreased the number of alcohol rewards obtained at the 2 highest doses,

ie 0.01 and 0.03 mg/kg p.o. (Figure 3). The LED [lowest effective dose] of bifeprunox significantly to inhibit operant responding for alcohol rewards = 0.01 mg/kg p.o. The effects of a single administration of bifeprunox (0.01 or 0.03 mg/kg p.o.) were very long-lasting being apparent 1.0h, 24h and 48h, but not 72h, after treatment.

Aripiprazole

As shown in Figure 4, rats trained to lever-press on an FR6 schedule to receive 6% ethanol solution rewards received between 9 - 11 rewards under baseline conditions. The number of ethanol rewards was not altered by oral administration of the drug vehicle (1% methylcellulose in water). Pretreatment with aripiprazole (0.03 or 0.1 mg/kg p.o.) 60 min before the start of the experimental session decreased the number of alcohol rewards obtained at the higher dose tested, ie 0.1 mg/kg p.o. (Figure 4). The LED [lowest effective dose] of aripiprazole significantly to inhibit operant responding for alcohol rewards = 0.1 mg/kg p.o. The effects of aripiprazole (0.1 mg/kg p.o.) were of short duration being apparent only 1.0h after treatment.

Conclusions

Acute administration of bifeprunox significantly decreased the number of alcohol rewards obtained by male Sprague-Dawley rats in an FR6 operant paradigm; LED = 0.01 mg/kg p.o. Moreover, the inhibitory effect of a single administration of bifeprunox (0.01 or 0.03 mg/kg p.o.) was very long-lasting being apparent 1.0h, 24h and 48h after treatment. Although aripiprazole also significantly decreased the number of alcohol rewards obtained, the dose required to produce this effect was much higher, ie LED = 0.1 mg/kg p.o., and its effect was of short duration being significant only 1.0h after treatment.

C. Operant responding for nicotine self-administration

Introduction

Bifeprunox, is a partial agonist at D_2 and D_4 receptors, that also binds D_3 receptors with even higher affinity than D_2 and D_4 receptors (Hesselink et al, 2003, Schiz. Res., 60:108). To explore the activity of bifeprunox as a potential treatment for nicotine abuse and dependence, its ability to attenuate the primary reinforcing properties of

nicotine was determined in rats that had been trained to self-administer nicotine intravenously.

Methods

Subjects: Ten male Wistar rats (Charles River, Italy) were used, weighing 250-275 g at the beginning of the experiment. They were housed individually at constant room temperature (21 ± 1°C) and relative humidity (60%) on a reversed light/dark schedule (light 7.30 p.m.- 7.30 a.m.) with food and water *ad libitum*. Animals were allowed to adapt to laboratory conditions for at least two weeks and handled for 5 min a day during this period. After this adaptation period, animals were food-restricted for the whole duration of the experiment to facilitate responding for the reinforcer. They received their food (20–25 g of standard laboratory chow) in the early evening at the conclusion of the nicotine self-administration training and over the preceding weekend. Animals on this food-restricted schedule remain healthy and gained weight at approximately 1 - 3 g/day.

Apparatus: All training and testing were conducted during the dark phase of the light/dark cycle at approximately the same time each day, and each rat was always exposed to the same operant chamber. Animals were trained and tested using standard rodent operant test chambers constructed from heavy-duty aluminium except for clear polycarbonate back, door and top, and equipped with two retractable levers. In half of the chambers the right-hand lever was designated as the "active" lever and in the other half the left-hand one. Approximately 15 g pressure was required to activate the lever and close the switch. The chambers had three lights, each 2.8 W, 24 V, one in the middle of the rear of the ceiling (the house light), and two on the front panel 6 cm above each lever. Auditory stimuli consisted of a 20 dB white noise above the background produced by a programmable audio generator presented through an 80 ohm speaker fitted in the centre of the back panel. When scheduled, 45 mg food pellets (Noyes improved formula A) were delivered from a dispenser to the magazine tray, which was accessible mid-way between the two levers. Intravenous infusions were administered by a syringe pump located inside the sound-attenuating cubicles. The experimental chamber was installed inside a sound-attenuating box, with an exhaust fan mounted on one side. Sound generator, stimulus lights, pellet dispenser

and syringe pump were controlled by an IBM compatible computer with MED software which also monitored input from the levers, recording the results of each experiment on files on the hard disk.

Chronic catheterisation of the jugular vein for nicotine self-administration: Catheters were made in-house using guide cannulae, silicon tubing (0.30 x 0.60 and 0.64 x 1.19 mm i.d. x o.d.), dental cement and silicon rubber as described by Caine et al (1993, Intravenous drug self-administration techniques in animals. In: Behavioral Neuroscience: A Practical Approach. Ed., Sahgal EA, pp 117-143, Oxford IRL Press, Oxford) with a few modifications (Cervo et al, 2003, Neuropsychopharmacology, 28: 1150-1159). Rats, anaesthetised with 5% isoflurane in N_2O/O_2 (70/30%) mixture and maintained with 1.5 to 2% isoflurane in the same mixture, were implanted with the proximal end of the catheter reaching the heart through the right jugular vein, continuing subcutaneously over the right shoulder, and exiting dorsally between the scapulae. During the six-day recovery period, rats received a twice daily subcutaneous injections of 75 mg/kg of Unasyn® (Ampicillin plus Sulbactam). Catheters were kept patent by daily intravenous infusions of 0.1 ml heparinised (30 units/ml) sterile 0.9% saline before and after each self-administration session. If a rat's behaviour during the self-administration training looked different from the normal baseline, the patency of the catheter was verified by injecting 0.05 ml i.v. of a solution containing 1.25 mg/ml midazolam maleate + 25 mg/ml ketamine hydrochloride. Using this technique, rats with patent catheters display clear signs of sedation within 3-s (Caine et al, 1999, J. Pharmacol. Exp. Ther., 291: 353-360).

Nicotine self-administration: Rats were trained according to the experimental procedure described by Cervo et al (2004, Psychopharmacology [Berl.],173: 124-31) except that nicotine was used instead of cocaine. As stated above, to facilitate acquisition of nicotine self-administration the rats were placed on a restricted diet for the whole duration of the experiment and initially trained to press the active lever for food pellets on a fixed-ratio 1 (FR1) schedule. The first session consisted of 30 min of non-contingent delivery of one 45 mg food pellet (Noyes improved formula A/I) every 30 s. In addition, each active lever-press delivered one food pellet. From the second 30-min session, food pellets were available on a continuous reinforcement schedule (i.e. each active lever press delivered one food pellet). As soon as rats

mastered this behaviour (generally in 2-3 days), the second inactive lever was introduced and the FR was increased to 2 and a time-out (TO) of 20 s was gradually imposed. Animals received a minimum of three 30 min food-training sessions under FR2/TO 20s in which they earned 50 food pellets. One week after catheter implantation, the nicotine self-administration was initiated. Rats were trained to i.v. self-inject (-)-nicotine hydrogen tartrate salt, 0.03 mg/0.1 ml/infusion in 2 s infusion, under a FR2 TO 20s for 1-h per day. These sessions started with extension of the active lever and concurrent presentation of a white noise (20 dB above background) that lasted throughout the session and served to differentiate the nicotine self-administration sessions from those in which food was available as the reinforcer. After each infusion, the lever remained inactive for 20 s to prevent accidental overdosing. This TO period was signaled by the cue light above the active lever coming on. From Day 5 of nicotine self-administration training, sessions started with extension of both the active and inactive levers and concurrent presentation of the white noise. During these sessions pressure on the active lever produced a nicotine infusion followed by a signalled 20-s TO period. Responses on the inactive lever were recorded, but had no programmed consequences. This training was conducted daily until nicotine-reinforced responding stabilized (± 15% over three consecutive training days).

Drug and treatment: To study the dose-response effect of bifeprunox a within-subject design was adopted in which each self-administration test session was followed by new self-administration sessions where rats reached new stable criterion until re-test. Rats were tested five times: after the injection of vehicle and after four doses of bifeprunox (0.004, 0.016, 0.064 and 0.25 mg/kg). Test sessions for each animal were separated by at least three self-administration days apart and to control for order effects different drug doses and the vehicle were administered in a random sequence across the self-administration sessions. Bifeprunox 0.004, 0.016, 0.064 and 0.25 mg/kg, dissolved in 10% w/v of 2-hydroxypropyl-β-cyclodextrin in sterile water, or vehicle, was given s.c. 30 min before testing. Drug solutions were freshly prepared immediately before use.

In the second part of the experiment, the effect of acute administration of bifeprunox (0.064 mg/kg s.c.) on the self-administration of a range of doses of nicotine, i.e. 0.005, 0.01, 0.03, 0.06 or 0.09 mg/0.1 ml/infusion, was determined.

Statistical Analyses: All data are expressed as the mean ± S.E.M. number of infusions, active and inactive lever-presses during the self-administration sessions. The mean number of nicotine infusions, "active" and "inactive" lever-presses were analysed by one-way ANOVA for repeated measures followed by the Newman-Keuls *post-hoc* comparison.

Results

The effects of acute injection of bifeprunox (0.004, 0.016, 0.064 and 0.25 mg/kg s.c.) on the number of infusions earned (upper panel), on the number of "active" (middle panel) and "inactive" (lower panel) lever-presses are shown in Figure 5. In this experiment, all rats rapidly developed a stable nicotine self-administration (mean ± S.E.M. number of days required to meet the training criterion were 13.0 ± 0.54 days). One-way ANOVA for repeated measures revealed a significant effect of bifeprunox on the number of infusion earned ($F(4,36)=9.3$, $P<0.01$) and on the number of "active" lever-presses ($F(4,36)=3.1$, $P<0.05$). No effect ($F(4,36)=2.3$, $P>0.05$)of the treatment was found on the number of "inactive" lever presses (Figure 5, lower panel). A significant reduction ($P<0.05$ vs. vehicle, Newman-Keuls test) of the number of infusions earned was found after 0.064 and 0.25 mg/kg bifeprunox (Figure 5, upper panel). No effect on the number of "active" lever-presses was found by *post-hoc* comparisons, although a clear tendency to reduce it was observed (Figure 5, middle panel).

The inhibitory effect of Bifeprenux (0.064 mg/kg s.c.) on self-administration of various doses of nicotine (0.005, 0.01, 0.03, 0.06 or 0.09 mg/0.1 ml/infusion) was also determined. As shown in Figure 6, acute administration of bifeprunox significantly ($P<0.05$) reduced nicotine self-administration across all of the doses tested (upper panel). Bifeprunox administration also significantly ($p<0.05$) decreased the number of presses on the "active" (middle panel), but not the "inactive" lever (lower panel).

Conclusions

The results of this experiment demonstrate that administration of 0.064 or 0.25 mg/kg s.c. Bifeprunox reduced the primary reinforcing properties of nicotine (0.03 mg/0.1 ml/infusion) as measured by the self-administration paradigm in rats. In

addition, bifeprunox (0.064 mg/kg s.c.) reduced self-administration of nicotine across a wide range of doses, i.e. 0.005, 0.01, 0.03, 0.06 or 0.09 mg/0.1 ml/infusion.

D. Expression of nicotine-induced sensitization

Introduction

One aspect of nicotine abuse is subjects frequently do not find the initial experience of taking this drug especially pleasurable; however, when the experience is repeated on subsequent occasions, the pleasurable properties of the drug become more manifest. It has been hypothesised that repeated exposure to various different types of substance of abuse pharmacologically sensitizes the brain to their reinforcing and rewarding actions and this is an important aspect of the process of developing psychological dependence. This process can be modelled in rats in which behavioural sensitization results in a progressive enhancement of locomotor activity following repeated intermittent drug administration. Sensitization can occur with almost all drugs of abuse, including nicotine, and is said to model some aspects of the development of drug abuse and dependence. The effect of bifeprunox to attenuate the expression of behavioural sensitization is predictive of its ability to attenuate some of the enhanced pleasurable subjective effects of the drug that underpin craving (psychological dependence) and demonstrate its potential as a drug as a treatment for nicotine abuse and dependence.

Methods

Subjects: In total, 48 male Wistar rats (HsdCpb:WU) were supplied by Harlan, Horst, The Netherlands. Bodyweight ranged between 261 and 319g at the start of the experiment. Animals were randomized over the groups to avoid bodyweight difference. The animals were housed in Makrolon Type III cages on sawdust bedding and a maximum of two animals per cage with free access to food and tap water. The cages and room were cleaned at regular intervals each week. The room was illuminated by fluorescent lights timed to give a 12 h light-dark cycle (on 06.00h, off 18.00h), the temperature range was 20°C to 22°C and the relative humidity range was 40% to 50%. The rats were kept in the rodent holding room for at least 7 days prior to being transferred to the test room for the study.

Experimental procedure: Locomotor activity was monitored and quantified using a "photobeam activity system" (PAS) for home cages. The measurements were performed in a chamber containing 24 perspex test cages (21x36x18 cm). These cages were placed between 7 photo-beams, which were arranged 4 cm above the chamber floor. During experimentation, counts of ambulatory movement and fine movements were integrated by the control and stored for subsequent off-line (statistical) analyses.

Nicotine-induced sensitization was established as follows: male Wistar rats received sensitizing regimes of nicotine (0.4 mg/kg s.c.) from Day 1 to Day 5. On Day 0 (baseline activity), Day 1 and Day 5 animals were placed in photo-beam chambers to measure locomotor activity. On Day 2 through to Day 4, animals received an injection with nicotine but were once again subjected to an injection of 0.4 mg/kg nicotine (sc) and locomotor activity was measured (challenge day). Before this nicotine injection, animals were injected with bifeprunox or aripiprazole to examine the ability of bifeprunox to attenuate/block the expression of nicotine-induced sensitization.

Drugs: The tests substances, bifeprunox and aripiprazole, were suspended in 1% methylcellulose. Nicotine ditartrate dehydrate was supplied by Acros Organics, Geel, Belgium and dissolved in 0.9% sterile saline.

Drug treatments: Nicotine (0.4 mg/kg) or saline was administered at a volume of 1 ml/kg s.c.. Animals received a total of either one (group A) or six (group B-F) injections of nicotine according to the protocol set out in Figure 7. Bifeprunox (0.01, 0.03 or 0.1 mg/kg), aripiprazole (0.3, 1.0 or 3.0 mg/kg), or vehicle (1% methylcellulose) was administered at a volume of 2 ml/kg once at t = -60 min before treatment with nicotine on the challenge day. When animals were reused treatments were separated by at least 7 days.

Statistical Analysis: Data are presented and analyzed as mean total ambulant movements over a period of 45 minutes after nicotine injection ± SEM. Data per day (Day 1, Day 5 or the challenge Day 10 or Day 17) were analyzed using a one-way ANOVA on dose followed by a *post-hoc* Bonferonni's adjustment where appropriate. The evaluation of activity scores between days were analyzed with a General Linear

Model (GLM) Repeated Measures test comparing the Day 1, Day 5 and the challenge day (being either Day 10 or Day 17), followed by a *post-hoc* Bonferonni's adjustment where appropriate. Data within each dose group were also analyzed by means of a paired samples t-test comparing Day 5 to the challenge day (being either Day 10 or Day 17). A p-value < 0.05 was considered to be statistically significant.

Results

Bifeprunox

As shown in Figure 8, repeated administration of nicotine (0.4 mg/kg s.c.) produced robust behavioural sensitization as revealed by an increase in the locomotor responses. Pretreatment with bifeprunox (0.01, 0.03 or 0.1 mg/kg p.o.) 60 min before nicotine (0.4 mg/kg s.c.) on the challenge day produced a dose-related inhibition of the expression of nicotine sensitization (Figure 8). The LED [lowest effective dose] of bifeprunox significantly to reduce the expression of nicotine-induced sensitization = 0.1 mg/kg p.o. The reduction in the expression nicotine-induced sensitization evoked by a dose of 0.03 mg/kg p.o. Bifeprunox just failed to reach statistical significance ($p = 0.05$).

Aripiprazole

As shown in Figure 9, repeated administration of nicotine (0.4 mg/kg s.c.) again produced robust behavioural sensitization as revealed by an increase in the locomotor responses. Pretreatment with aripiprazole (0.3, 1.3 or 3.0 mg/kg p.o.) 60 min before nicotine (0.4 mg/kg s.c.) on the challenge day did not significantly inhibit the expression of nicotine sensitization (Figure 9).

Conclusions

Acute administration of bifeprunox produced a dose-related inhibition of the expression of nicotine sensitization in male Wistar rats; LED = 0.1 mg/kg p.o. In contrast, Aripiprazole did not significantly reduce the expression of nicotine sensitization in this model.

E. Cue-induced re-instatement of operant responding for nicotine self-administration (nicotine craving)

Introduction

The pleasurable subjective and reinforcing effects of drugs are generally linked with contextual cues associated with the misuse of legal or illegal substances of abuse, *eg* nicotine and smoking in social settings or after a meal. Because of their association with the specific substance of abuse, the presentation or occurrence of such cues can be strong motivators for the continued use/misuse of the abused drug, and if the subject tries to quit its use, they can also induce craving and relapse during abstinence. A drug that reduces craving for a legal or illegal substance of abuse will help subjects in their efforts to quit and to prevent relapse during the abstinence phase. These effects can be modelled in rats trained to lever-press for nicotine in a 2-lever operant paradigm (1 lever is assigned to the delivery of the substance of abuse [active] and presses result in injections of a small dose of nicotine, whilst pressing on the other lever [inactive] does not result in the delivery of nicotine rewards. The self-administration of the strong reinforcer, nicotine, is paired with a contemporaneous contextual cue, *eg* a light or a tone signal, whilst vehicle is paired with a different cue. By linking the pleasurable subjective and reinforcing effects of nicotine with the cue, presentation of the cue initiates "craving" and motivates pressing activity on the active lever. After a period of extinction when the rat is denied access to the substance of abuse and its paired cue, re-presentation of the cue that was paired with nicotine self-administration, but not nicotine itself, reveals the degree of craving for the drug that is quantified by the number of presses on the active lever that previously delivered the nicotine rewards.

Methods

Subjects: Eight male Sprague-Dawley CD®IGS rats (Charles River, Italy) were used, weighing 250-275 g at the beginning of the experiments. They were housed individually at constant room temperature (21 ± 1°C) and relative humidity (60%) under an inverted light/dark schedule (lights on between 19.30h - 07.30h) with food and water ad libitum. Animals were allowed to adapt to laboratory conditions for at least two weeks and handled for 5-min a day during this period. After this adaptation period, to facilitate responding for reinforcer, animals were

food-restricted for the whole duration of the experiment and received their food (20-25 g of standard laboratory chow) in the early evening, at the conclusion of the nicotine self-administration training and over the preceding week-end. Animals on this food restricted schedule remain healthy and gain weight at approximately 1 - 3 g/day, yet they were highly motivated to respond in operant cages.

Catheters were made in-house using guide cannulae, silicon tubing, dental cement and silicon rubber according to Caine et al. (1993, Intravenous drug self-administration techniques in animals. In Behavioral Neuroscience: A Practical Approach. Ed. Sahgal EA. pp 117-143, Oxford IRL Press, Oxford) with a few modifications (Cervo et al., 2003, Neuropsychopharmacology, 28:1150-1159). All training and testing were conducted during the dark phase of the light/dark cycle at approximately the same time each day, and each rat was always exposed to the same operant chamber. Animals were trained and tested using standard rodent operant test chambers and equipped with two retractable levers. In half the chambers the right-hand lever was designated as the active lever, in the others the left-hand one. The chambers had three lights, each 2.8 W, 24 V, one in the middle back of the ceiling (the house light), and two on the front panel 6 cm above each lever. Auditory stimuli was a 20 dB white noise above the background produced by a white noise generator or a 7 kHz, 70 dB intermittent tone generated by a tone source, both presented through an 80-ohm speaker fitted in the centre of the back panel. Intravenous infusions were administered by a syringe pump. Rats were trained according to the experimental procedure described by Cervo et al. (2003) except that nicotine instead of cocaine was used. The experimental procedure involved training the rats to self-administer IV nicotine while simultaneously establishing discriminative stimuli associated with, and predictive of, nicotine availability or non-availability. As stated above, to facilitate acquisition of nicotine self-administration the rats were placed on a restricted diet (20 g/day rat chow for the whole duration of the experiment) and trained to press a lever for food pellets on a fixed ratio 1 (FR1) schedule.

Drugs: Bifeprunox (Batch number 2038301; 0.004, 0.016, 0.064 and 0.25 mg/kg), dissolved in 1 ml 10% β-cyclodextrin in sterile water, or vehicle, was injected subcutaneously (s.c.) 30 min before testing. Drug solutions were freshly prepared immediately before use.

Drug treatment: To study the dose-response effect of bifeprunox a within-subject design was adopted in which each reinstatement test session was followed by new extinction sessions until re-test. Rats were tested five times with nicotine-associated stimuli, after the injection of vehicle and after four doses of bifeprunox. Animals were also tested one time under saline-associated cue, after injection of vehicle. Test sessions for each animal were separated by at least three extinction days apart and to control for order effects, different drug doses and the vehicle were administered in a random sequence across reinstatement sessions.

Statistical Analysis: All data are expressed as the mean ± S.E.M. number of active lever presses during the self-administration, extinction and reinstatement phases. The number of nicotine infusions earned during the last three days of self-administration was analyzed by one-way analysis of variance (ANOVA) for repeated measurements. Also the numbers of lever presses during the last three days of saline self-administration and the last three days of extinction between the different reinstatement sessions were also analyzed separately as above. Since there were no differences between sessions, the last 3 d for each condition were pooled for further statistical analyses. Thus, the effects of bifeprunox on reinstatement induced by nicotine-associated stimuli to revive seeking behavior were analyzed by one-way ANOVA with repeated measurements, with the test sessions as the main factor. Whenever a significant effect was found, post-hoc comparisons were done using the Newman-Keuls test.

Results

Reinstatement

Reintroduction of the nicotine-associated cues led to immediate recovery of operant responding that was significantly higher than that observed either after introduction of the saline-associated stimuli or the 3 preceding extinction sessions (both $P<0.01$, Newman–Keuls test). The overall behavioural output after presentation of nicotine-associated cues was similar to that observed during nicotine self-administration and was significantly different from saline self-administration ($P<0.01$, Newman–Keuls test). The number of lever-presses during the presentation of saline-associated stimuli did not differ from the 3 preceding extinction sessions. Because the rats had to meet the extinction criterion, lever-presses during the

extinction sessions preceding the reintroduction of the nicotine- and saline-associated cues did not differ significantly between groups.

Bifeprunox

Figure 10 shows the effect of bifeprunox on the reinstatement of active lever pressing induced by reintroduction of the stimuli associated with nicotine compared with saline non-reinforced. Reintroduction of nicotine associated stimuli increased the number of stimuli earned as compared with introduction on non-reward associated stimuli ($p<0.01$, Newman-Keuls test). bifeprunox pretreatment significantly modified the number of stimuli earned ($F(13,91) = 11.0$, $p<0.01$, one-way ANOVA). *Post-hoc* comparison by Newman-Keuls test revealed that bifeprunox 0.016, 0.064 and 0.25 mg/kg, but not 0.004 mg/kg bifeprunox, significantly reduced the number of cues earned during the 1 hr reinstatement session ($P<0.01$ vs vehicle-treated group). The LED [lowest effective dose] of bifeprunox = 0.016 mg/kg p.o.

As a consequence of reduced salience of nicotine associated stimuli, Bifeprunox pretreatment significantly modified the number of lever presses after nicotine-associated stimuli reintroduction on the active ($F(13,111)=10.6$, $P<0.01$, one-way ANOVA for repeated measurement) but not on the inactive lever ($F(13,111)=1.4$, $P>0.05$, one-way ANOVA for repeated measurement).

Conclusions

In a cue-induced relapse model to determine the effect of bifeprunox on nicotine-induced craving, bifeprunox dose-dependently reduced cue-induced operant responding that had previously been paired with access to nicotine rewards with a LED = 0.016 mg/kg p.o. In summary, bifeprunox reduced nicotine craving.

F. Operant responding for the consumption of sucrose pellets

Introduction

Bifeprunox, is a partial agonist at D_2 and D_4 receptors, that also binds D_3 receptors with even higher affinity than D_2 and D_4 receptors (Hesselink et al., 2003). It is well known that highly palatable foods, *eg* carbohydrates, fats, are both hedonistic and reinforcing. Food addiction has been likened to substance use disorder (Corwin & Grigson, 2009, J. Nutrition, 139: 617-619; Ifland et al., 2009, Med. Hypotheses, 72:

518-526). To establish whether bifeprunox modifies operant responding maintained by a powerful food reinforcer, *ie* sucrose pellets.

Methods

Subjects: Twelve male Wistar rats (Charles River, Italy) were used, weighing 250-275 g at the beginning of the experiments. They were housed individually at constant room temperature (21 ± 1°C) and relative humidity (60%) under a reversed light/dark schedule (light 7.30 p.m.- 7.30 a.m.) with access to food and water *ad libitum*. Animals were allowed to adapt to laboratory conditions for at least two weeks and were handled for 5 min each day during this period.

After this adaptation period, to maintain the same experimental procedure used in the nicotine experiments (see Example C), animals were food-restricted for the whole duration of the experiment and received their food (20–25 g of standard laboratory chow in the early evening, at the conclusion of the operant training sessions and over the preceding weekend. Animals on this food restricted schedule usually remained healthy and gained weight at a rate of ~1–3 g/day.

Apparatus: All training and testing was conducted during the dark phase of the light/dark cycle at approximately the same time each day, and each rat was always exposed to the same operant chamber. Animals were trained and tested using standard rodent operant test chambers (ENV-007, MED Associates Inc., St. Albans, VT) constructed from heavy-duty aluminium except for clear polycarbonate back, door and top, and equipped with two retractable levers. In half of the chambers, the right-hand lever was designated as the active [rewarded] lever, in the others the left-hand one. Approximately 15 g pressure was required to depress the lever and close the switch. The chambers had three lights, each 2.8 W, 24 V, one in the middle rear of the ceiling (the house light), and two on the front panel 6 cm above each lever. Auditory stimuli consisted of a 20-dB white noise above the background produced by a programmable audio generator, presented through an 80-ohm speaker fitted in the centre of the back panel. When scheduled, 45 mg food pellets were delivered from a dispenser to the magazine tray, which was accessible mid-way between the two levers. The experimental chamber was installed inside a sound-attenuating box, with an exhaust fan mounted on one side. The sound generator, stimulus lights and pellet dispenser were controlled by an IBM compatible computer with MED software which

also monitor input from the levers, recording the results of each experiment on files on the hard disk.

Sucrose pellet-reinforced operant responding: Rats were trained according to the experimental procedure described by Paterson et al. (2003, Psychopharmacology, 167: 257-264). As stated above, to maintain the same experimental conditions used in the nicotine experiments the rats were placed on a restricted diet [20-25 g/day rat chow for the whole duration of the experiment] and initially trained to press a lever for food pellets on a fixed ratio 1 (FR1) schedule.

The first session consisted of 30 min of non-contingent delivery of one 45-mg food pellet every 30 s. In addition, each active lever press delivered one food pellet. From the second 30-min session, food pellets were available on a continuous reinforcement schedule. As soon as rats mastered this behaviour (generally in 2-3 days), the second inactive lever was introduced and the FR increased to 2; at the same time, a time-out (TO) of 20 s was gradually imposed. Animals received a minimum of three 30 min food-training sessions under FR2-TO 20 s in which they earned at least 50 food pellets.

After the initial lever-pressing for food, the reinforcer was changed to 45 mg sucrose pellets available under a FR2-TO 20s schedule for 30 min. These sessions started with extension of both active and inactive levers and concurrent presentation of a white noise (20 dB above background) that lasted throughout the session and served to differentiate the sucrose sessions from the food sessions. After each sucrose pellet, the active [rewarded] lever remained inactive for 20 s. This TO period was signalled by the cue light above the active lever coming on. This training was conducted daily until sucrose pellets responding stabilized (± 15% over three consecutive training days).

Drug and treatment: To study the dose-response effect of bifeprunox on operant responding for sucrose pellets a within-subject design was adopted in which each self-administration test session was followed by new self-administration sessions where rats reached new stable criterion until re-test. Rats were tested five times: after the injection of vehicle and after four doses of bifeprunox (0.004, 0.016, 0.064 and 0.25 mg/kg, s.c.). Test sessions for each animal were separated by at least three days and to control for order effects different drug doses and the vehicle were administered in a random sequence across the self-administration sessions. Bifeprunox 0.004,

0.016, 0.064 and 0.25 mg/kg, dissolved in 10% w/v of 2-hydroxypropyl-β-cyclodextrin in sterile water, or vehicle, was given subcutaneously (s.c.) 30 min before testing. Drug solutions were freshly prepared immediately before use.

Statistical Analyses: All data are expressed as the mean ± S.E.M. for the number of pellets earned, and for the number of "active" and "inactive" lever-presses made during the sessions of sucrose-maintained operant behaviour. The results were analysed by one-way ANOVA for repeated measures followed by the Newman-Keuls *post-hoc* comparison.

Results

Figure 11 shows the effects of bifeprunox on the number of sucrose pellets earned (upper panel), on the number of lever-presses on the "active" [rewarded lever] (middle panel) and "inactive" [non-rewarded lever] (lower panel). In this experiment, all rats developed stable reinforced responding for sucrose pellets. One-way ANOVA for repeated measures revealed a significant effect of bifeprunox on the number of sucrose pellets earned ($F(4,44)=62.8$, $P<0.01$) and on the number of "active" ($F(4,44)=28.9$, $P<0.01$), but not "inactive" lever-presses ($F(4,44)=2.2$, $P<0.05$). *Post-hoc* comparisons by Newman-Keuls test showed a significant reduction in the number of sucrose pellets earned after administration of 0.064 and 0.25 mg/kg bifeprunox ($P<0.01$ compared to vehicle) (Figure 11, upper panel). A significant reduction of the number of "active" lever-presses was also found after bifeprunox ($P<0.01$ for 0.064 and 0.025 mg/kg, and $P<0.05$ for 0.016 mg/kg compared to vehicle, Newman-Keuls test) (Figure 11, middle panel). None of the doses of Bifeprunox significantly altered the number of "inactive" lever-presses.

Conclusions

The results clearly demonstrate that sucrose is a powerful reinforcer in rats as revealed by the observation that the animals were prepared to perform substantial numbers of lever-presses (work) to obtain sucrose pellets. Bifeprunox significantly decreased the reinforcing effect of sucrose as shown by dose-dependent reductions in the numbers of sucrose pellets earned and lever-presses on the "active" [rewarded] lever; lowest effective doses (LEDs) of bifeprunox were 0.064 and 0.016 mg/kg, sc, respectively.

This effect was highly specific because bifeprunox treatment did not alter the number of lever-presses on the "inactive" [non-rewarded] lever.

G. Cue-induced re-instatement of operant responding for sucrose pellets (sucrose craving)

Introduction

It is universally recognised that the consumption of highly palatable foods is both hedonistic and reinforcing and this has led to the hypothesis that people can become addicted to specific foods ("food addiction") in the same way that humans become addicted to substances of abuse (Corwin & Grigson, 2009, J. Nutrition, 139: 617-619; Ifland et al., 2009, Med. Hypotheses, 72: 518-526; Davis & Carter, 2009, Appetite, 53: 1-8). Like any other addiction, food addiction has adverse effects on health and well-being through over-consumption, obesity and related metabolic disorders (Corwin & Grigson, 2009; Ifland et al., 2009; Davis & Carter, 2009). Sucrose is a powerful reinforcer and it has been reported that this refined nutrient can cause addiction (Avena et al., 2008, Neurosci. Biobehav. Rev., 32: 20-39).

The inhibition of drug craving (psychological dependence) is considered to be a promising strategy to treat addiction because it may reduce the risk of relapse - without the necessity to interfere directly with the central reward system. The pleasurable and reinforcing effects of indulging in the consumption of highly palatable foods ("forbidden foods") or taking drugs of abuse are often linked with contextual cues. Because of their association with the specific pleasurable experience, the presentation or occurrence of such cues can be strong motivators to consume the specific palatable food, and in addition, the presentation of such cues can induce craving and relapse during abstinence. These effects can be modelled in rats by pairing the reinforcer, *ie* sucrose pellets, together with a recognisable cue, *eg* a light or a tone. In this paradigm, rats are trained to lever-press for sucrose in a 2-lever operant paradigm (1 lever is assigned to the delivery of sucrose pellets ["active"] and presses result in injections of a sucrose pellet contingent with the presentation of a contextual cue, *eg* light. Pressing on the other lever ["inactive"] is not rewarded and is associated with the presentation of a different cue, *eg* an auditory tone. When responding for the sucrose reinforcer is stable, the reinforcer and the stimulus cues are withdrawn and lever-pressing gradually subsides ("extinction").

The objective of the present study was to evaluate the efficacy of bifeprunox to modulate sucrose seeking behavior induced by re-introduction of cues associated with this nutritional reward (sucrose pellets) to determine whether bifeprunox would be of benefit in the treatment of food addiction and bingeing.

Methods

Subjects: Thirty male Wistar rats (Charles River, Calco, Italy) were used, weighing 200-225 g at the beginning of the experiments. They were housed individually at constant room temperature (21 ± 1°C) and relative humidity (60%) under a reversed light/dark schedule (light 7.30 p.m.- 7.30 a.m.) with food and water available *ad libitum*. Animals were allowed to adapt to laboratory conditions for at least two weeks and were handled for 5 min a day during this period. After this adaptation period, to facilitate responding for reinforcement and to maintain the same experimental procedure used in the nicotine experiments, animals were food-restricted for the whole duration of the experiment and received their food (20-25 g of standard laboratory chow) in the early evening, at the conclusion of the operant training sessions and over the preceding week-end. Animals on this food restricted schedule generally remained healthy and gained weight at approximately 1-3 g/day, yet they were highly motivated to respond in operant cages.

Experimental apparatus: All training and testing were conducted during the dark phase of the light/dark cycle at approximately the same time every day, and each rat always used the same operant chamber. Animals were trained and tested using standard rodent operant test chambers constructed from heavy-duty aluminium except for clear polycarbonate back, door and top, and equipped with two retractable levers. In half the chambers the right-hand lever was designated as the "active" lever [rewarded lever], in the others it was the left-hand one. Approximately 15 g pressure was required to depress the lever and close the switch. The chambers had three lights, each 2.8 W, 24 V, one in the centre rear of the ceiling (the house light), and two on the front panel 6 cm above each lever. Auditory stimuli consisted of 20-dB white noise above the background produced by a white noise generator or a 7-kHz, 70-dB intermittent tone generated by a tone source, both presented through an 80-ohm speaker fitted in the centre of the rear panel. Each experimental chamber was installed inside a sound-attenuating box, with an exhaust fan mounted on one side. Sound

generator, stimulus lights and pellet dispenser were controlled by an IBM-compatible computer with MED software which also monitored input from the levers, recording the results of each experiment on files on the hard disk.

Sucrose-reinforced behavioural training - operant sessions and conditioning: Rats were trained according to the experimental procedure described by Cervo et al. (2007, Int. J. Neuropsychopharmacol. 10: 167-181) with some modifications to the method. The experimental procedure involved training the rats to earn sucrose pellets while simultaneously establishing discriminative stimuli respectively associated with, and predictive of, the availability or non-availability of sucrose pellets. As stated above, to facilitate acquisition of maintained lever-pressing for sucrose pellets and to employ the same experimental conditions that were used in the nicotine experiments, the rats were placed on a restricted diet for the whole duration of the experiment and trained to press a lever for food pellets on a fixed ratio 1 (FR1) schedule.

The first session consisted of 30 min of non-contingent delivery of one 45-mg food pellet every 30 s. In addition, each lever press delivered one food pellet. From the second 30 min session, food pellets were available on a continuous reinforcement schedule. As soon as rats mastered this behavior, the second "inactive" lever [non-rewarded lever] was introduced, the FR was increased to 2 and a time-out (TO) of 20 s was gradually imposed. Animals received a minimum of three 30 min food-training sessions under FR2-TO 20 s in which they earned at least 50 food pellets.

After the initial lever pressing for food, the reinforcer was changed to 45 mg sucrose pellets available under a FR2-TO 20s schedule for 30 min 5 days a week. These sessions started with extension of the active lever and concurrent presentation of a white noise (20 dB above background) that lasted throughout the session and served as the discriminative stimulus (S^{D+}) for sucrose pellets availability. After each sucrose pellet, the lever remained inactive for 20 s. This TO period was signalled by the cue light above the active lever coming on. From day 5 of training, sessions started with extension of both levers and concurrent presentation of S^{D+}. During these sessions, pressure on the active lever was reinforced by a sucrose pellet followed by a signalled 20-s TO period.

From day 7, a second daily 30 min session was introduced during which sucrose pellets were not available. These sessions begun with extension of both levers and

simultaneous illumination of the house light that remained on for the duration of the session and served as a discriminative stimulus for sucrose pellets non-availability (S^{D-}). Each response on the active lever resulted in the triggering of the feeder motor for 0.5 s and the presentation of an intermittent tone (7 KHz, 70 dB) as a TO cue for 20 s, during which time the lever remained non-operative. Responses on the inactive lever were recorded, but had no programmed consequences. Thus from day 8, rats were placed on a "discrimination learning" regimen that comprised two daily 30 min sessions, separated by 1 h resting in the home cage, when sucrose pellets were either available or unavailable as a reinforcer using a random sequence. During this phase, the S^{D+} and S^{D-} continued to be presented throughout the sessions, and sucrose pellets and non-rewards were always followed by the signalled 20-s TO period. The S^{D}s were not turned off during the TO periods. Responses on the inactive lever were recorded, but had no programmed consequences. This training was conducted daily until sucrose pellets responding stabilized (± 15% over 3 consecutive training days).

Extinction: After meeting the above training criterion, rats were placed under extinction conditions until the end of the experiment. Sessions began with the extension of both levers without presentation of S^{D}s. Responses on the previously active lever resulted in 0.5 s activation of the feeder motor, but had no other programmed consequences. Responses on the inactive lever were also recorded. One daily 1-h extinction session was conducted, until the criterion of ≤ 15 responses per session over 3 consecutive days was reached.

Reinstatement: Reinstatement tests began 1 day after each animal had met the extinction criterion. Tests lasted 1 h during which rats were exposed to the S^{D+} or S^{D-} under conditions identical to the discrimination learning phase, except that sucrose pellets were not available. In both conditions, responses on the previously active lever was followed by 0.5 s activation of the feeder motor and a 20-s signaled TO period during which both levers remained inactive.

Drugs and treatment: It has previously been reported that the ability of the cue associated with the sucrose pellet reinforcer to reinstate sucrose-seeking behaviour diminishes (Baptista et al., 2004, J. Neurosci. 24: 4723-4727; Cervo et al., 2007, Int. J. Neuropsychopharmacol. 10: 167-181). For this reason, a between-subjects design

was used in which each rat underwent 2 test sessions, first in the presence of S^{D+} and the second in the presence of S^{D-} to evaluate whether bifeprunox influenced the seeking behaviour for this food reward. Test sessions were followed by new extinction sessions until re-test. Rats were randomly divided in four groups of 7-8 to be treated with vehicle, or bifeprunox 0.016, 0.064 and 0.25 mg/kg, sc. The order of the two test session was counterbalanced in each group so that half of the animals were first tested after reintroduction of the cues associated with the sucrose pellets and the second half after reintroduction of the stimuli associated with no rewards.

Bifeprunox 0.016, 0.064 and 0.25 mg/kg, dissolved in a solution of 1 ml 10% w/v of 2-hydroxypropyl-β-cyclodextrin in sterile water, or vehicle was given subcutaneously (s.c.) 30 min before testing. Fresh drug solutions were prepared immediately before use.

Statistical Analysis: All data are expressed as the mean number lever-presses ± SEM on the active or inactive lever during the operant sessions, extinction and reinstatement phases. The number of sucrose pellets earned during the last 3 days of reinforced training were analysed by one-way analysis of variance (ANOVA) for repeated measurements. Also, the number of lever-presses performed during the last 3 days of conditioning for the non-availability of sucrose pellets were analysed separately as above. Since there were no differences between sessions, the last 3 days for each condition results were pooled for further statistical analyses. Thus, the effects of bifeprunox on the reinstatement of sucrose-seeking behavior induced by the reintroduction of the stimuli associated with sucrose pellets as a reinforcer were analysed by mixed factorial ANOVA for repeated measurements, with the test sessions as the main factor. Whenever a significant effect was found, *post-hoc* comparisons were made using Tukey's test.

Results

Reinstatement

As reported in Figure 12, reintroduction of the cue associated with sucrose pellets as a reinforcer led to immediate reinstatement of operant responding that was significantly higher than after introduction of the stimuli associated with no sucrose rewards and the 3 preceding extinction sessions (both $p<0.01$, Tukey's test). The overall behavioral output after presentation of sucrose-associated cues was similar to those

observed in sessions where responding was reinforced by sucrose pellets. They were also significantly different from responding on the non-rewarded "inactive lever ($p<0.01$, Tukey's test). The number of lever-presses during the presentation of the non-reward-associated stimuli did not differ from the 3 preceding extinction sessions. Because the rats had to meet the extinction criterion, lever-presses during the extinction sessions preceding the reintroduction of the stimuli associated with sucrose reinforcement and non-reward did not differ between groups. These results indicated that the reintroduction of the discriminative stimuli associated with, and predictive of, operant responding previously rewarded by the delivery of sucrose pellets robustly reinstated operant responding in the absence of further reinforcement demonstrated a robust and reliable sucrose-seeking behavior. This behavioral effect cannot be attributed to non-specific arousal or spontaneous activity because responding on the inactive lever remained negligible and, more importantly, responding after presentation of cues associated with no rewards remained at the extinction level in all of the test sessions.

Bifeprunox

Figure 12 shows the effect of bifeprunox on the number of sucrose-seeking cues earned during reinstatement induced by reintroduction of the stimuli associated with sucrose pellets compared to its effect on operant responding elicited by stimuli associated with no reward ($p<0.01$, Tukey's test). Bifeprunox pretreatment significantly modified the number of cues earned ($F_{treatment}(3,26)=3.7$, $p<0.01$; $F_{test\ session}(3,78)= 68.7$, $p<0.01$ and $F_{treatment\ x\ test\ session}(3,78)=10.8$, $p<0.01$, two-way ANOVA). *Post-hoc* comparisons by Newman-Keuls test revealed that bifeprunox 0.016, 0.064 and 0.25 mg/kg, s.c. significantly reduced the number of cues earned during the 1 h reinstatement session ($p<0.01$ vs. vehicle-treated group) even though they were still higher than the number of non-rewarded cues earned.

Conclusions

In this experimental paradigm, bifeprunox significantly attenuated sucrose-seeking behaviour induced by the reintroduction of cues associated sucrose pellets as a reinforcer. The LED (lowest effective dose) was 0.016 mg/kg, sc.

The effect on sucrose pellets seeking behavior could not be attributed to drug-induced disruption of behavior because responses on the inactive lever were not affected.

DISCUSSION

In the first aspect of this invention, it has been surprisingly demonstrated that bifeprunox has the potential to treat substance use disorder, which is abuse and/or dependence of a wide variety of psychoactive drugs and other substances, *eg* stimulants (cocaine, amphetamine and MDMA ["ecstasy"]), sedatives (benzodiazepines, barbiturates, opioids and cannabis) and hallucinogens (LSD, phencyclidine, ketamine and "magic mushrooms"), and legal substances of abuse, *eg* alcohol and nicotine. In particular the use of bifeprunox to treat nicotine abuse and dependence, alcohol abuse and dependence, or the combination of nicotine and alcohol abuse and dependence.

Drug abuse and dependence is a complex phenomenon comprising various distinct components, which will be described briefly, that ultimately result in the excessive use of the drug in question. For a drug be abused, the experience when using it must be pleasurable (reinforcement); the more pleasurable the effect of the drug, the more likely it is to be abused. Sensitization, which is a greater pharmacological effect of a drug after it has been used on several occasions, or the development of tolerance generally lead to escalating drug use in subjects. Craving which is the irresistible desire to take the drug again (psychological dependence) or the occurrence of unpleasant physical signs during drug withdrawal, *eg* sweating, tremors, cramps or convulsions (physical dependence), are also powerful drivers of both continued excessive drug use or relapse on drug withdrawal. Finally, drug use can be a ritualised or social experience that is associated with a specific context, smoking a cigarette with coffee or after a meal, or set of cues, *eg* the smell of tobacco smoke in clubs or pubs or smoking by others in social settings. The presentation of these cues is also a strong motivator to increase drug use by subjects, or to relapse to drug taking when attempting to abstain or withdraw from using the particular drug. In this invention, animal models have been used that have explored the therapeutic benefit of bifeprunox by determining its effects on all of the major aspects of treating drug abuse and dependence.

This aspect of the invention is supported by findings obtained with bifeprunox in animal models that are predictive of clinical efficacy of drugs in the treatment of nicotine abuse and dependence, *ie* reducing the operant responding of rats

intravenously to self-administer a range of doses of nicotine (reducing the reinforcing effects of nicotine and/or decreasing the motivation to take nicotine), inhibition of the expression of nicotine sensitization (reducing nicotine addiction and or nicotine tolerance) and prevention of cue-induced re-instatement of nicotine seeking (nicotine craving). Results are also presented to demonstrate that bifeprunox will be efficacious in the treatment of alcohol abuse and dependence, *ie* this drug reduced the 24h *ad libitum* consumption of an 8% alcohol solution (drug will help to reduce excessive alcohol consumption) and inhibited operant responding to consume a 6% alcohol solution (reducing the reinforcing effects of alcohol and/or decreasing the motivation to consume alcohol).

These results were surprising and inventive for the following reasons:

1. Aripiprazole, another drug that is from the same pharmacological class, *ie* a dopamine D_2-like / 5-HT_{1A} receptor partial agonist, did not replicate the beneficial effects of bifeprunox in the animal models of nicotine or alcohol abuse/addiction. These findings for aripiprazole are consistent with a report that this drug was failed to demonstrate benefit in another preclinical model of alcohol abuse and dependence (Ingman et al., 2006). The predictive validity of the animal models is also supported by the failure of aripiprazole to show clinical benefit in a large well powered placebo controlled trial to determine its efficacy in the treatment of alcohol addiction (Anton et al., 2008).

2. In the only model of nicotine abuse/addiction where bifeprunox and aripiprazole were both found to be active, bifeprunox was not only 10x more potent than aripiprazole but it also had a very much longer duration of action.

3. The advantaged profile of bifeprunox relative to aripiprazole cannot be explained by potency differences between the compounds in the rat as demonstrated by their relative effects to inhibit conditioned avoidance responding (a rat model predictive of antipsychotic properties).

Bifeprunox and aripiprazole share almost identical pharmacological profiles as 3rd generation dopamine D_2-like / 5-HT_{1A} receptor partial agonists. As shown in the

Table below, the receptor affinities of these two 3rd generation antipsychotic drugs are very similar.

Primary pharmacology of bifeprunox and aripiprazole			
Neurotransmitter	Receptor subtype	Bifeprunox D_2/5-HT_{1A} partial agonist (pK_i)	Aripiprazole D_2/5-HT_{1A} partial agonist (pK_i)
Dopamine	D_1	6.0	6.1
	D_{2S}	8.5	8.3
	D_3	9.1	8.4
	$D_{4.4}$	8.0	7.0
	D_5	6.0	6.1
Serotonin (5-hydroxytryptamine)	5-HT_{1A}	8.2	8.1
	5-HT_{2A}	6.1	7.8
	5-HT_{2C}	< 6.0	7.3

The similarity of the dopamine and serotonin receptor binding affinities of bifeprunox and aripiprazole are also supported by data reported in the scientific literature (see review by Newman-Tancredi et al, 2007, Curr Opinion in Invest Drugs 8, 539-554). Moreover both drugs have similar intrinsic efficacy as partial agonists of both D_2 receptors (Cosi et al, 2006, Eur J Pharmacol535, 135-144) and 5-HT_{1A} subtypes (Newman-Tancredi et al, 2005, Int J Neuropsychopharmacol 8, 341-356).

Conditioned avoidance responding (CAR) is tested in a 2-compartment shuttle-box where the presentation of a specific cue, *eg* a light or tone, signals to the rat that if the animal does not move to the other chamber, it will be punished by receiving a mild foot-shock. Antipsychotic drugs of all classes impair conditioned avoidance responding without causing sedation and this model is well established as a test to detect novel antipsychotic drugs. Bifeprunox and aripiprazole are both active in the CAR model, but there is around a 10-fold potency difference between bifeprunox and aripiprazole. This potency difference is likely to be due to pharmacokinetics as demonstrated by the similar levels of D_2 receptor occupancy at their respective LEDs (see Table below taken from data on file at Solvay Pharmaceuticals).

LEDs (lowest effective doses) for efficacy in conditioned avoidance testing together with percentage occupancy of D_2 receptors in the brain		
3rd generation antipsychotic drug	CAR (LED mg/kg)	D_2 occupancy (LED in CAR)
Bifeprunox	0.25	>90%
Aripiprazole	2.5	>90%

Bifeprunox was active and highly potent in all of the animal models predictive of clinical efficacy for the treatment of nicotine abuse and dependence, ie reduction of nicotine self-administration, inhibition of the expression of nicotine sensitization and prevention of cue-induced re-instatement of nicotine seeking (nicotine craving), and also animal models predictive of clinical efficacy in the treatment of alcohol abuse and dependence, ie reduction of 24h *ad libitum* consumption of an 8% alcohol solution and inhibition of operant responding to consume a 6% alcohol solution. These findings were surprising and novel because in spite of the similarity between the pharmacological profiles of bifeprunox and aripiprazole, the latter was found to have no inhibitory effect on the expression of nicotine sensitization or on the *ad libitum* 24h consumption of an 8% alcohol solution even when tested at doses much greater than 10x the LED of bifeprunox. Moreover, in the model of alcohol abuse and dependence where aripiprazole was found to be active, *ie* inhibition of operant responding for the consumption of a 6% alcohol solution, the efficacy of bifeprunox was much greater, ie 10-fold more potent, and with significant efficacy out to 48h after a single administration, it also has a much longer duration of action. These findings are summarised in the Table below and would not be predicted on the basis of the known pharmacological profiles of bifeprunox and aripiprazole.

Substance use disorder model	Bifeprunox (LED)	Aripiprazole (LED)	Potency difference
Ad libitum 24h consumption of alcohol	0.01 mg/kg	Inactive ≤ 6.0 mg/kg	> 600x
Operant responding to access alcohol consumption	0.01 mg/kg Duration of action - significant effect @ 1h, 24h and 48h	0.1 mg/kg Duration of action - significant effect @ 1h	≥ 10x @ 1h + greater duration of action ≥ 48h
Expression of nicotine sensitization	0.1 mg/kg	Inactive ≤ 3 mg/kg	> 30x
LED = Lowest effective dose			

In the second aspect of this invention, it has also been it has been surprisingly demonstrated that bifeprunox has the potential to treat food addiction and bingeing. As discussed elsewhere in the filing, food addiction shares many symptoms in common with substance use disorder as defined in the DSM-IV TR®. Bifeprunox was surprisingly found to reduce the reinforcing effects of sucrose which was used as a typical highly palatable, high calorie, "forbidden foods" linked to food addiction. The results predict that bifeprunox will reduce the motivation to binge on "forbidden foods" and/or decrease their rewarding properties that lead to their excessive consumption in food addiction. Bifeprunox also reduced the reinstatement of cue-induced sucrose seeking behaviour predicting that it will also be effective in treating food cravings. Through this action bifeprunox will help cure patients of their addiction to "forbidden foods", and in addition, will help maintain abstinence when patients try to reduce or abstain from eating them.

In addition to having surprising efficacy in the treatment of substance use disorder based on its pharmacology, this therapeutic benefit was also unexpected because of the observation that the beneficial effects of bifeprunox were observed in rat models of drug abuse and dependence, and food addiction/bingeing at doses that

were 2.5 to 25-fold lower than its LED (lowest effective dose) in the rat CAR model of antipsychotic efficacy.

Comparison of the relative potency of bifeprunox in models of abuse and dependence compared with its antipsychotic action			
Rat model	Therapeutic indication	LED (lowest effective dose) (mg/kg)	Potency difference
CAR (conditioned avoidance responding)	Antipsychotic efficacy	0.25	Not applicable
Ad lib alcohol consumption	Alcohol abuse and dependence	0.01	25x
Operant responding to consume alcohol	Alcohol abuse and dependence	0.01	25x
Operant responding to self-administer nicotine	Nicotine abuse and dependence	0.064	4x
Expression of nicotine sensitisation	Nicotine abuse and dependence	0.1	2.5x
Cue-induced relapse to nicotine seeking	Nicotine abuse and dependence	0.016	15x
Operant responding to consume sucrose pellets	Food addiction and bingeing	0.064	4x
Cue-induced relapse to sucrose seeking	Food addiction and bingeing	0.016	15x

In rat models, bifeprunox has been shown to be effective in treating various components not only of drug abuse and dependence, but also food addiction/bingeing. Although these 3 compounds, ie nicotine, alcohol and sucrose have very different pharmacological and physiological properties, the one common feature is that they are highly rewarding and potentially addictive. From this observation, 2 conclusions can be drawn:

1. Bifeprunox will be efficacious in treating the combination of nicotine and alcohol abuse and/or dependence.
2. Bifeprunox will be efficacious in treating not only nicotine and/or alcohol abuse and/or dependence, but also the abuse and/or dependence relating to other legal and illegal substances of abuse.

3. Bifeprunox will be efficacious in treating food addiction/bingeing not only of sucrose, but also of other highly palatable, calorie-dense "forbidden foods" implicated in this disorder.

The findings can be summarised as follows. In the first aspect of this invention, bifeprunox has surprisingly been found to be exceptionally potent and efficacious in the treatment of substance use disorder, and in particular treating nicotine abuse and dependence, alcohol abuse and dependence or the combination of nicotine and alcohol abuse and dependence.

In the second aspect of this invention, bifeprunox has surprisingly been found to be exceptionally potent and efficacious in the treatment of food addiction/bingeing, and in particular treating food addiction/bingeing for sugary or sweet foods or drinks.

These discoveries support the use of bifeprunox as a method of treatment for substance use disorder and/or food addiction/bingeing, the use of bifeprunox or pharmaceutically acceptable salts thereof as an ingredient in a medicament for the treatment of substance use disorder and/or food addiction/bingeing, and the use of bifeprunox or pharmaceutically acceptable salts thereof in the manufacture of a medicament for the treatment of substance use disorder and/or food addiction/bingeing.

CLAIMS

1. Bifeprunox for use in the treatment of a patient with a substance use disorder and/or food addiction.

2. Bifeprunox of claim 1, wherein the substance is a psychoactive substance selected from alcohol, tobacco (nicotine), stimulants, sedatives, euphoriants, entactogens and hallucinogens.

3. Bifeprunox of claim 2, wherein the psychoactive substance is alcohol.

4. Bifeprunox of claim 2, wherein the psychoactive substance is tobacco (nicotine).

5. Bifeprunox of any one of claims 1-4, wherein the substance use disorder is selected from substance abuse or substance dependence.

6. Bifeprunox of claim 1, wherein the food is highly palatable and calorie dense.

7. Bifeprunox of claim 5, wherein the highly palatable and calorie dense food contains sucrose or another mono- or polysaccharide as a nutrient.

8. Bifeprunox of any one of claims 1, 6-7, wherein the food addiction is selected from food bingeing, food dependence or food craving.

9. Bifeprunox of any one of claims 1-8 wherein the dose is selected from 0.5-12 mg.

10. Bifeprunox of claim 9, wherein the dose is selected from 2-8 mg.

11. Bifeprunox of any one of claims 1-10, wherein bifeprunox is in the form of a pharmaceutically acceptable salt.

12. Bifeprunox according to claim 8, wherein it is in the form of a mesylate salt.

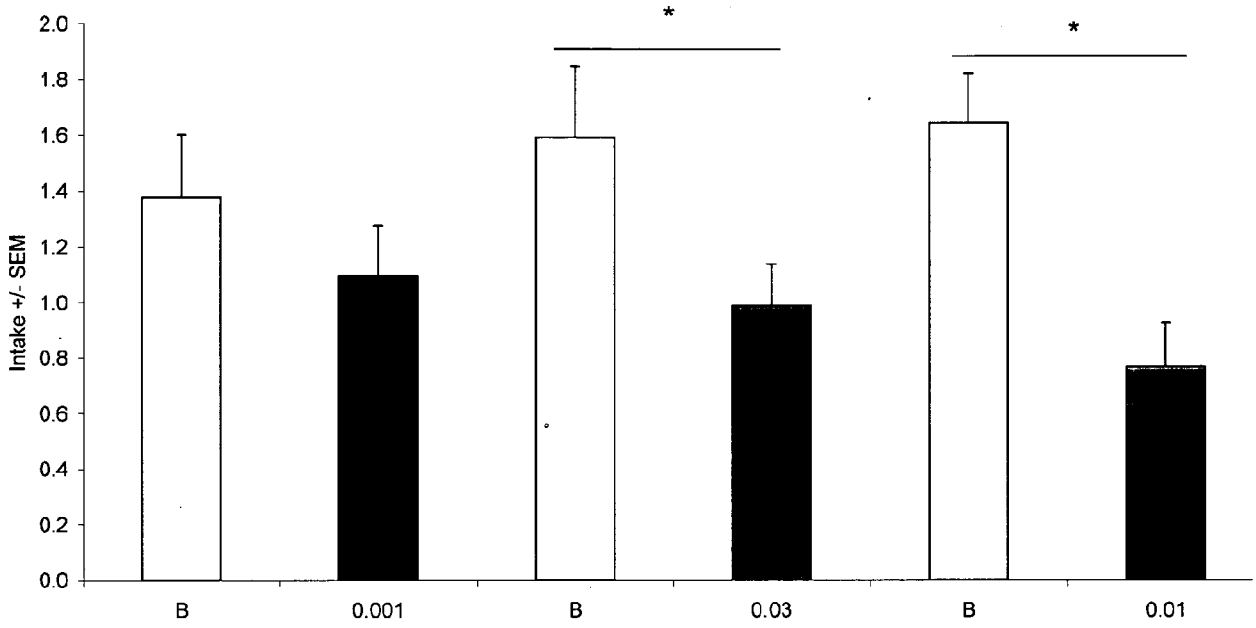
13. Bifeprunox according to claim 9, wherein it is in the form of a crystalline salt.

14. Bifeprunox of claim 10, wherein the salt is the crystal form alpha.

15. Use of bifeprunox for the preparation of a medicament for the treatment of a patient with a substance use disorder and/or food addiction.

Figure 1 Inhibition by acute administration of bifeprunox of *ad libitum* (non-operant) 24h consumption of alcohol by male Sprague-Dawley rats – Attenuation of alcohol consumption (substance use)

Bifeprunox



Vehicle (1% MC)

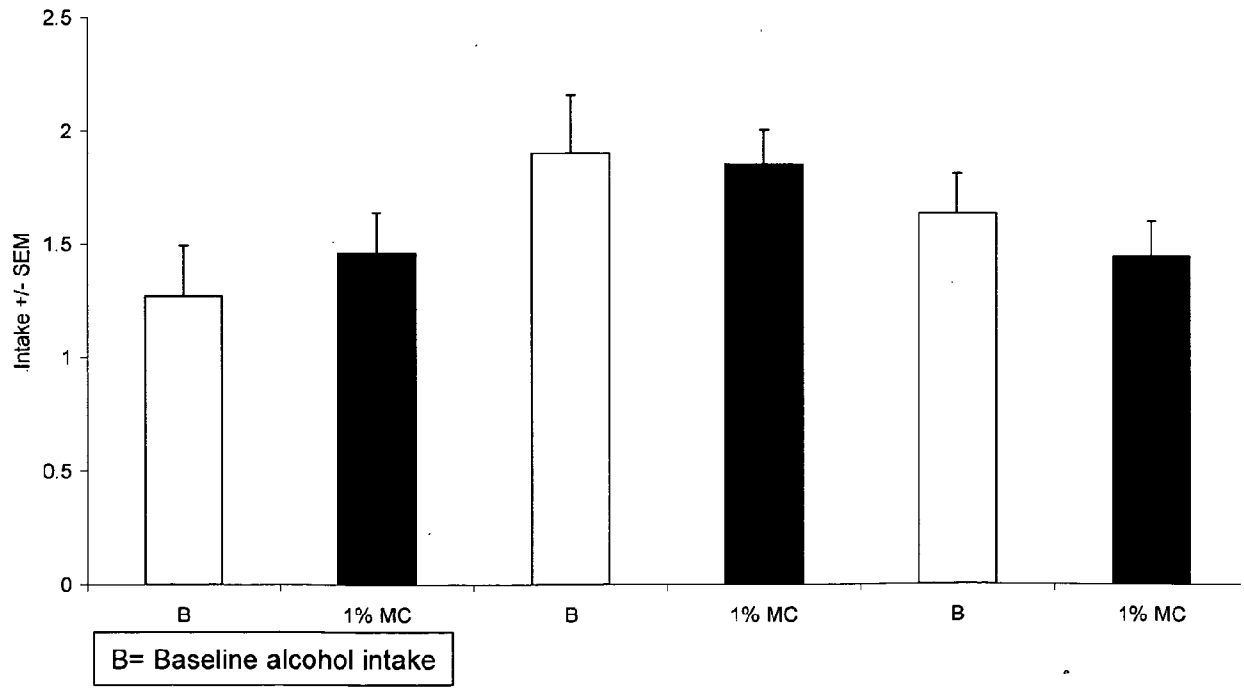


Figure 2 Lack of effect of acute administration of aripiprazole on the *ad libitum* (non-operant) 24h consumption of alcohol by male Sprague-Dawley rats – Absence of effect on alcohol consumption (substance use)

Aripiprazole

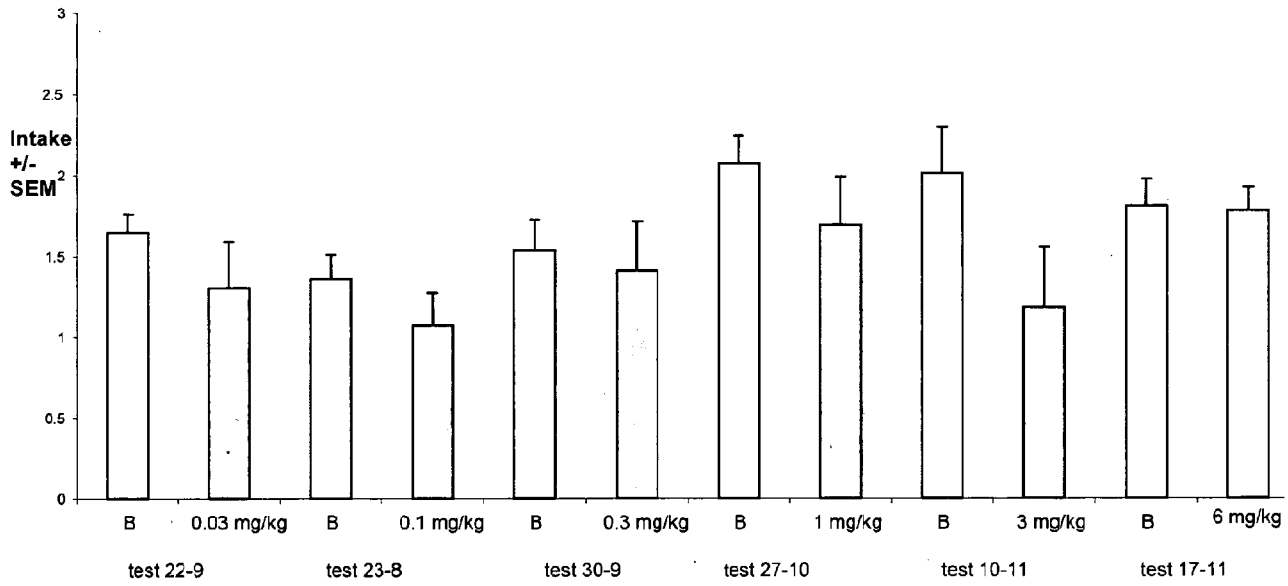


Figure 2 – continued

Vehicle (1% MC)

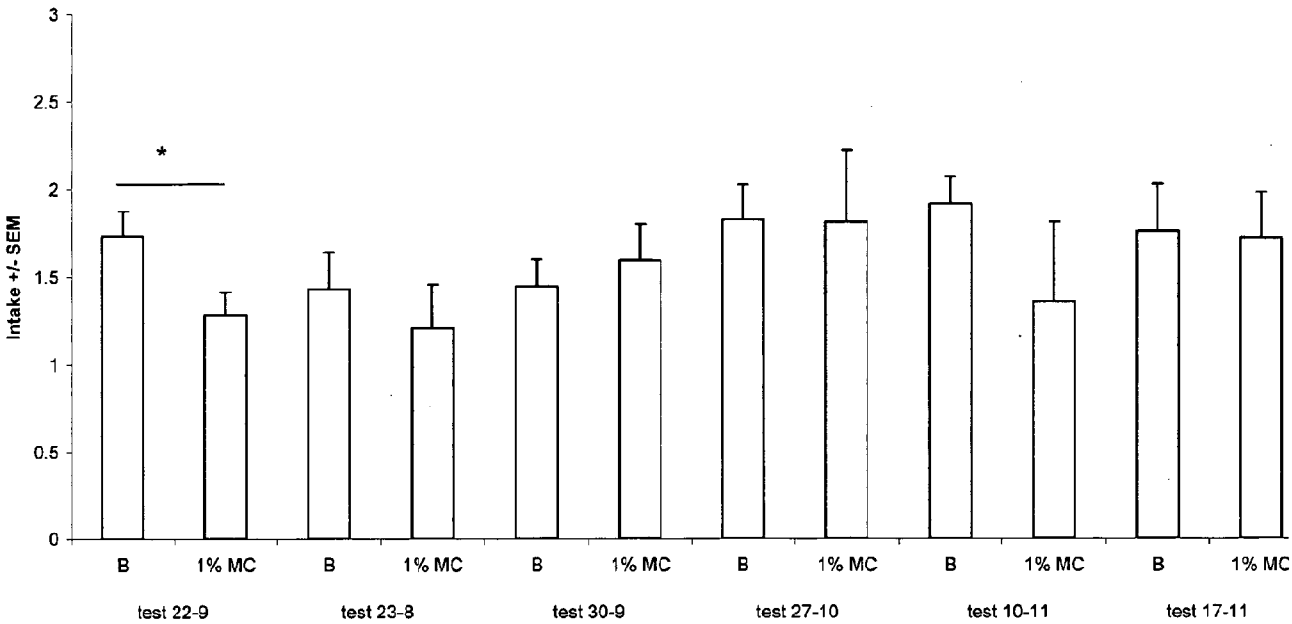
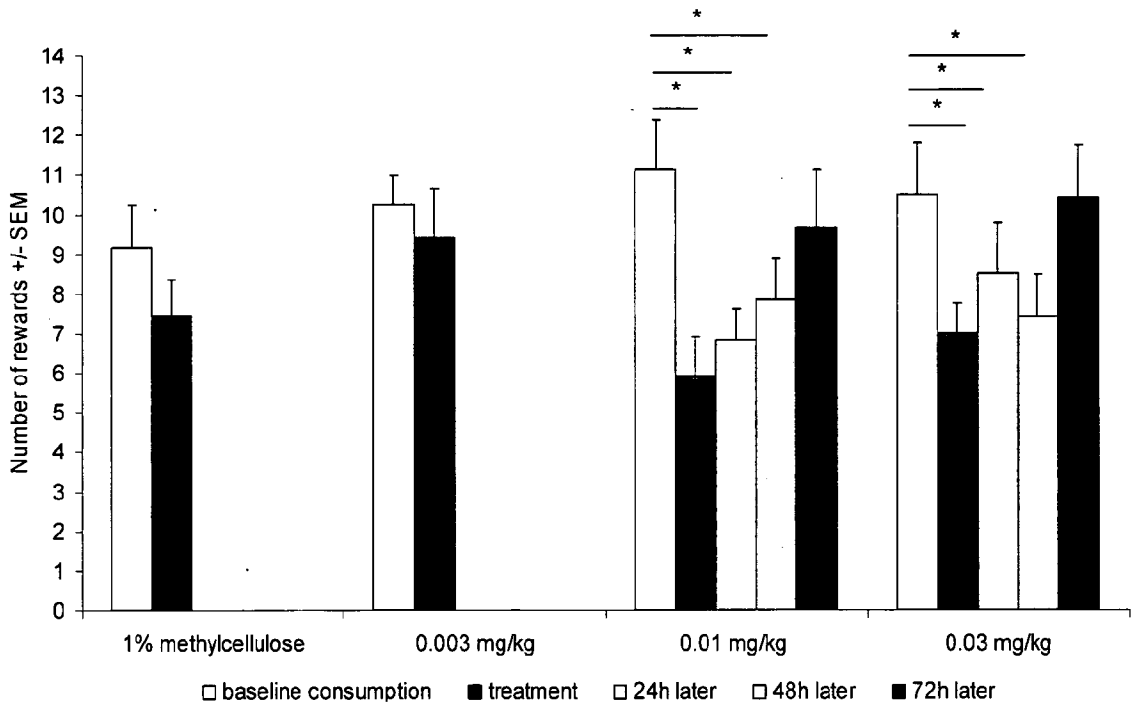
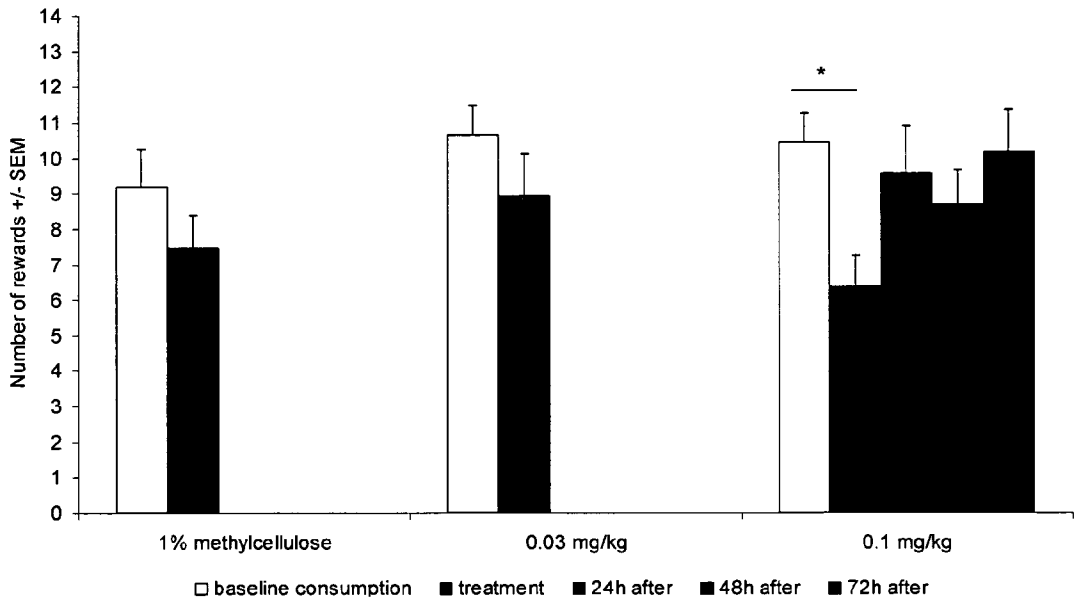


Figure 3 Inhibition by acute administration of bifeprunox of operant (lever-press) responding for access to alcohol consumption by Sprague-Dawley rats - Attenuation of the reinforcing effects of alcohol



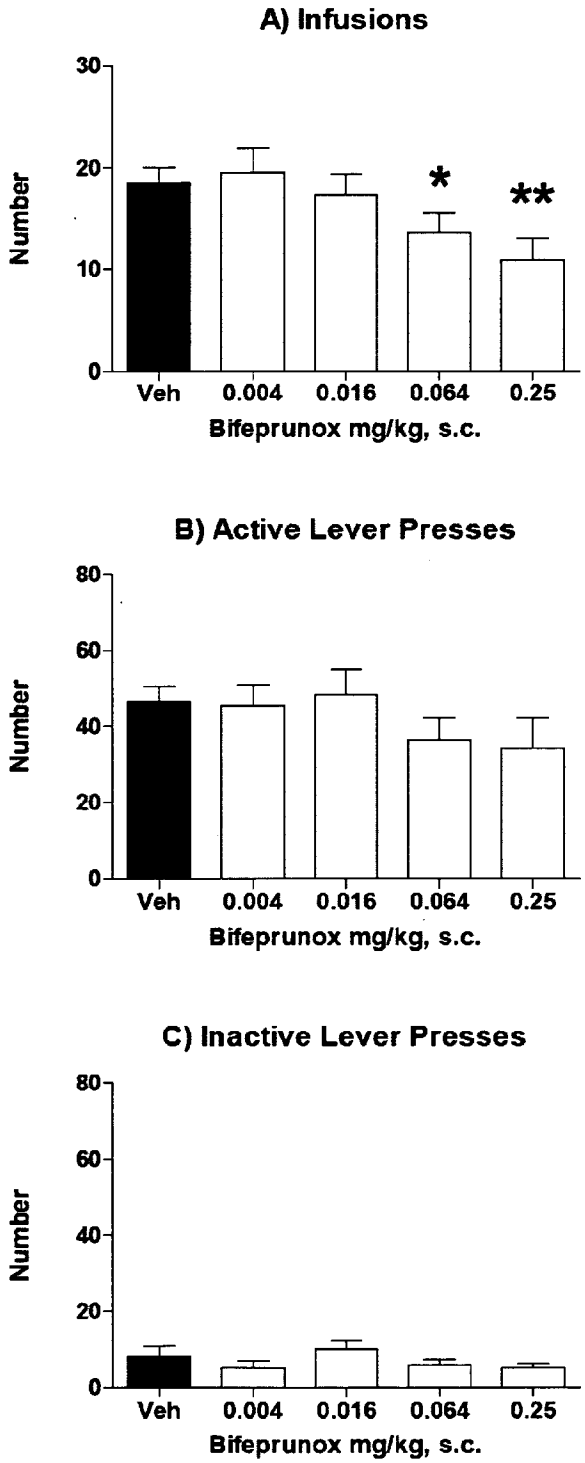
Lowest effective dose (LED) = 0.01 mg/kg

Figure 4 Effect of acute administration of aripiprazole on operant (lever-press) responding for access to alcohol consumption by Sprague-Dawley rats - Attenuation of the reinforcing effects of alcohol



Lowest effective dose (LED) = 0.1 mg/kg

Figure 5 Effect of acute administration of bifeprunox on the number of nicotine infusions obtained, and on the numbers of "active" and "inactive" lever-presses - Attenuation of nicotine consumption (substance use)



*P < 0.05 and **P<0.01 vs. respective vehicle, Newman-Keuls test

Figure 6 Effects of acute administration of bifeprunox (0.064 mg/kg s.c.) or vehicle on self-administration of various doses of nicotine - Attenuation of nicotine consumption (substance use)

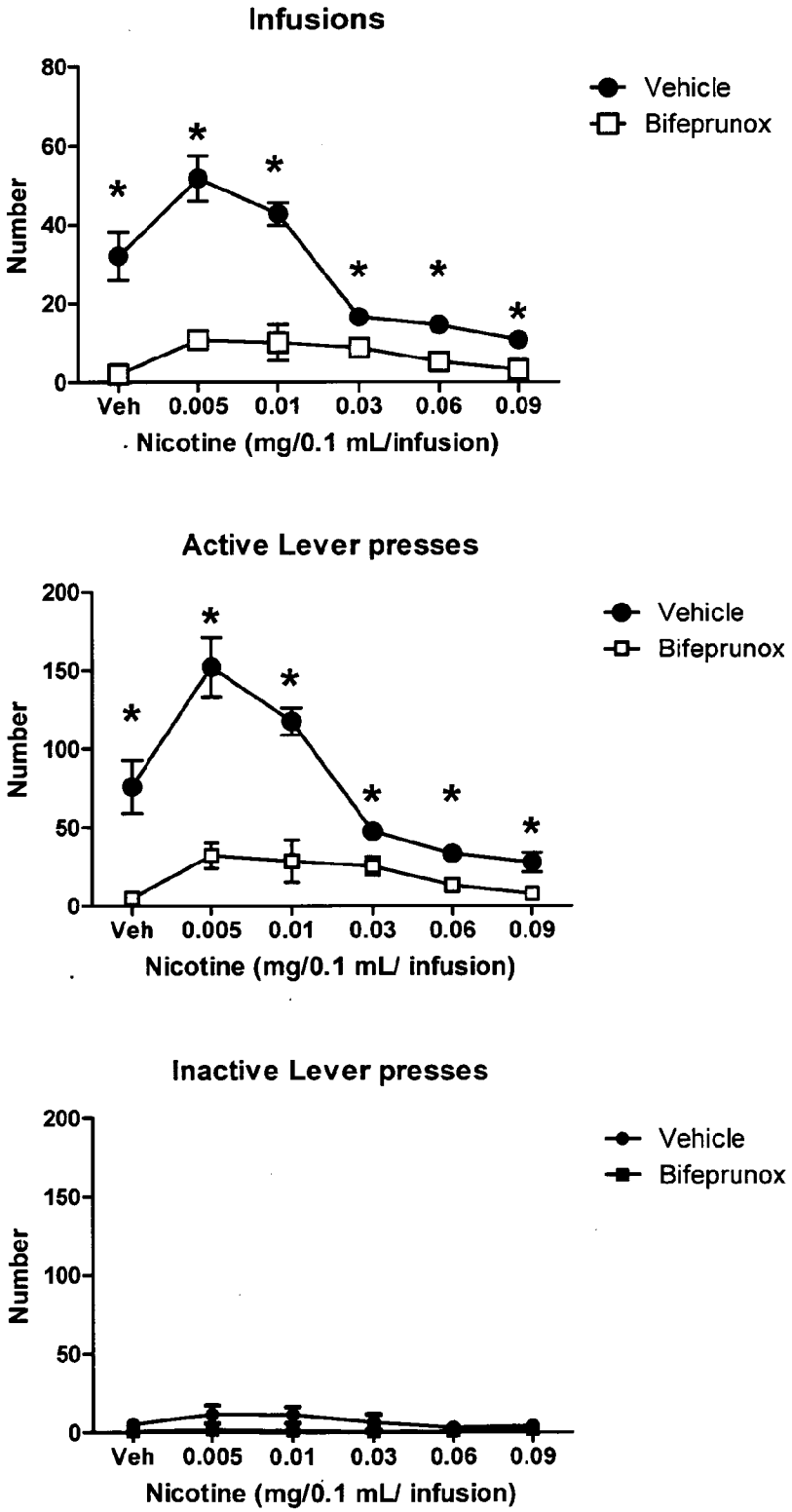
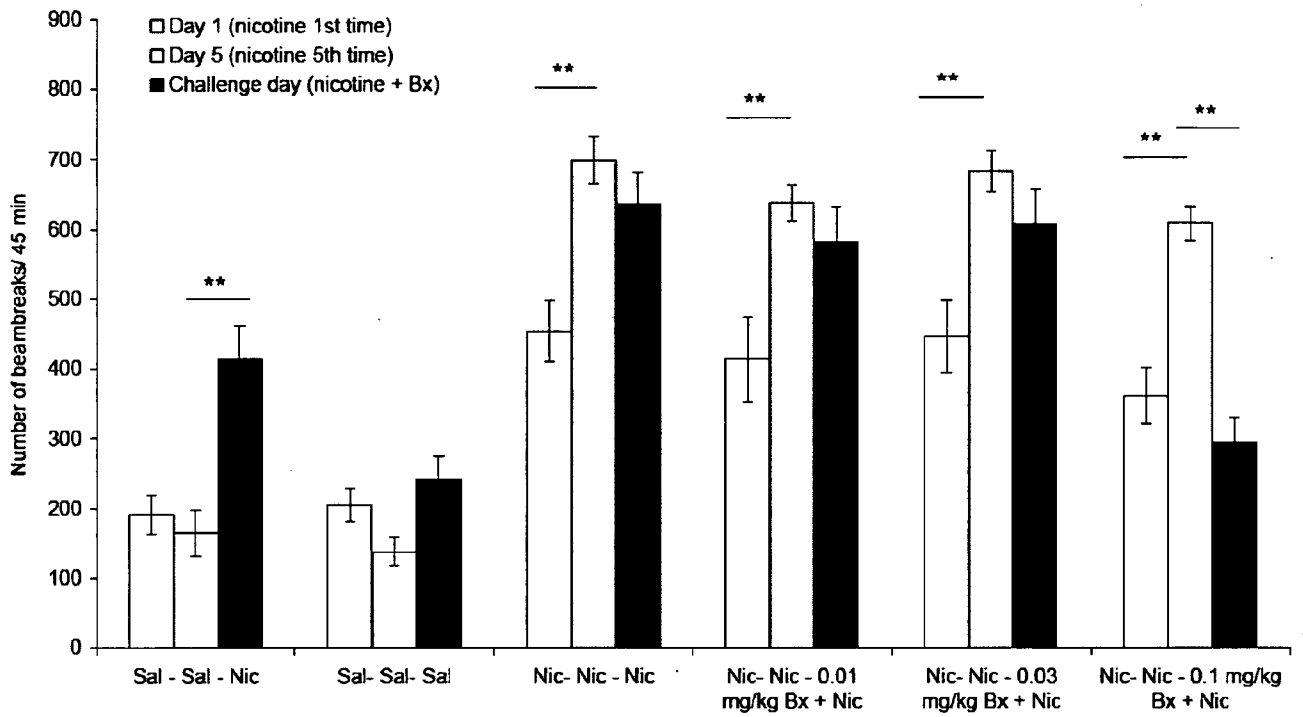


Figure 7 Treatment protocol for experiments to investigate the effect of bifeprunox or aripiprazole on nicotine-induced sensitization in male, Wistar rats.

	Days 0	1	2	3	4	5	6	7	8	9	10	17
treatment	SAL	NIC	NIC	NIC	NIC	NIC				SAL	NIC	NIC
activity measurement	x	x				x				x	x	x
Bifeprunox/Aripiprazole											x	x

Figure 8 Inhibition by acute administration of bifeprunox of the expression of nicotine-induced sensitization in male, Wistar rats - Attenuation of nicotine addiction (substance addiction)



Lowest effective dose (LED) = 0.1 mg/kg

Figure 9 Lack of effect of acute administration of aripiprazole on the expression of nicotine-induced sensitization in male, Wistar rats – Lack of effect on nicotine addiction (substance addiction)

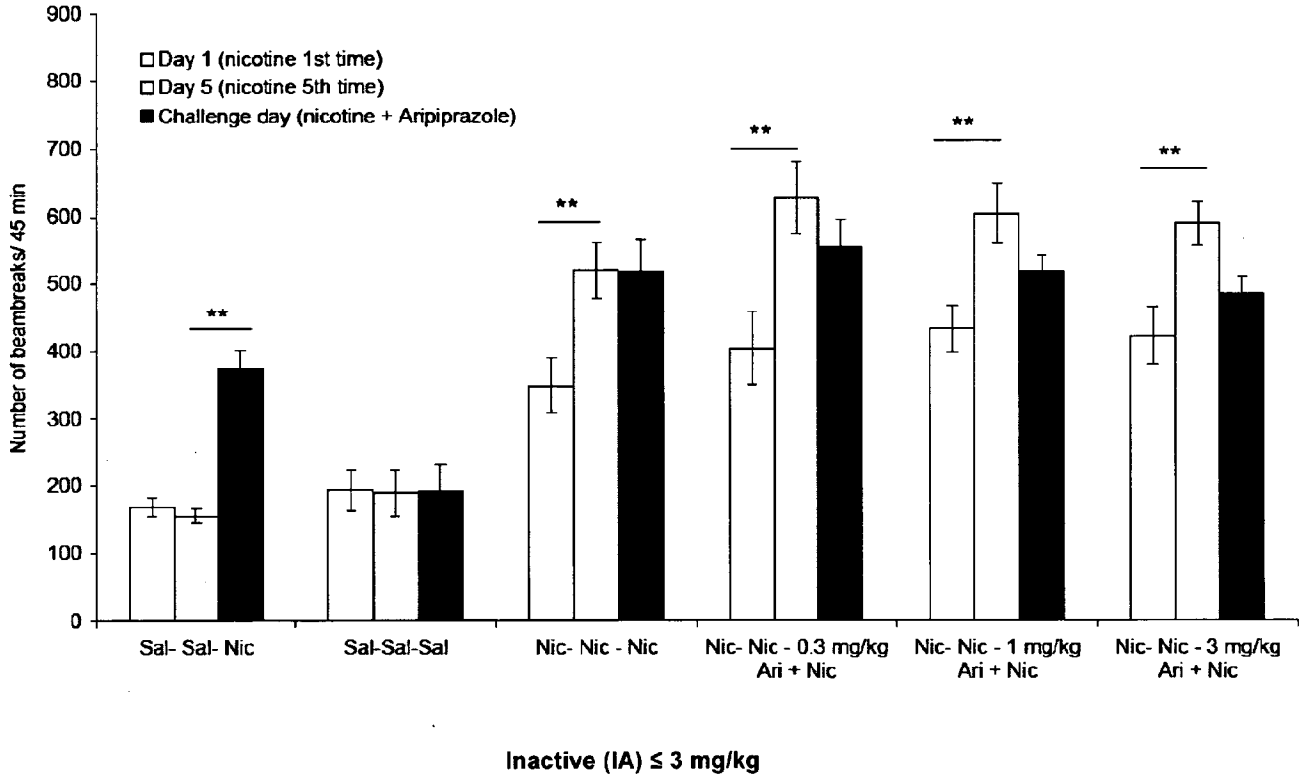
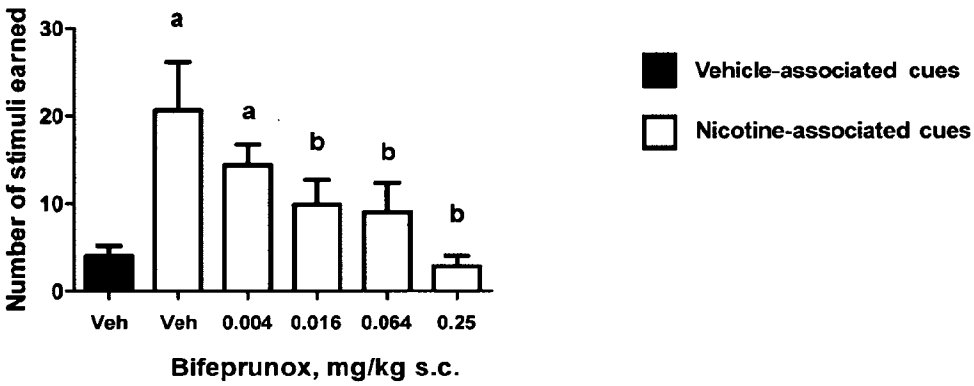


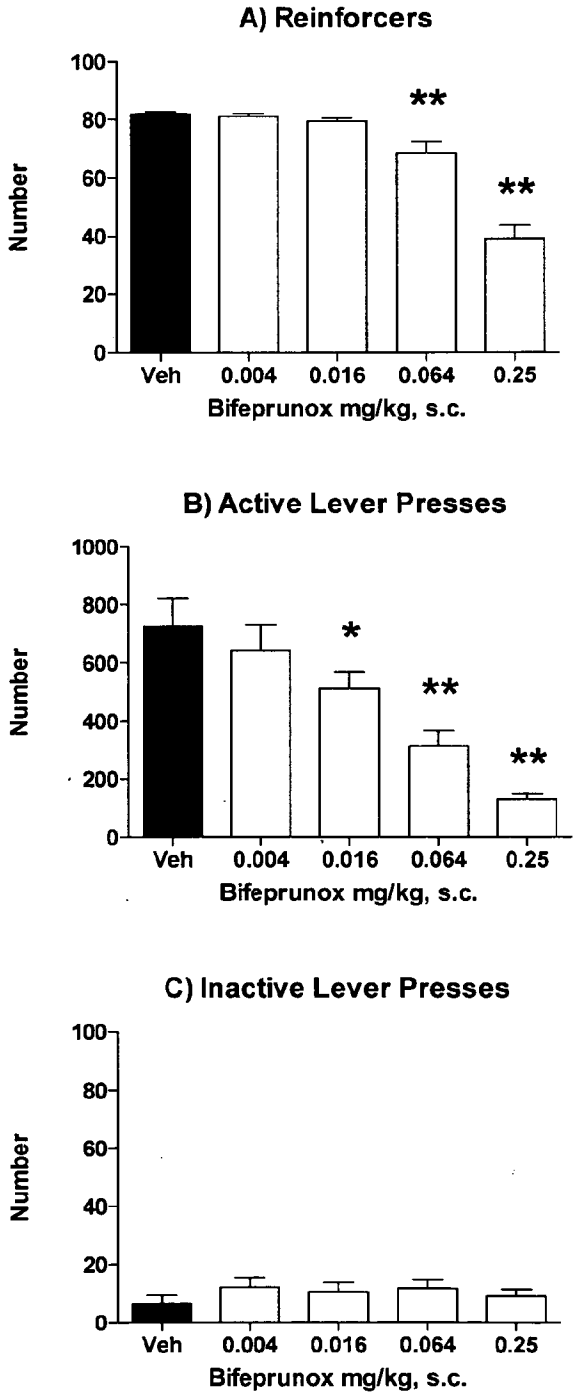
Figure 10 Prevention by acute administration of bifeprunox of the cue-induced reinstatement of nicotine seeking in male Sprague-Dawley rats - Attenuation of the nicotine craving (substance craving or psychological dependence)



[a] p <0.05 vs. vehicle-associated cues
[b] p <0.05 vs. nicotine-associated cues

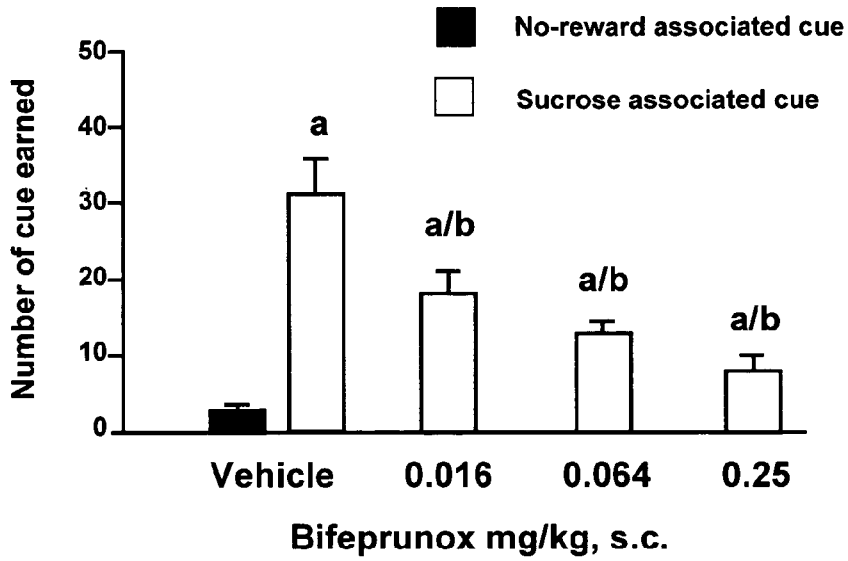
Lowest effective dose (LED) = 0.016 mg/kg, sc

Figure 11 Effect of acute administration of bifeprunox on the number of sucrose pellets earned, and on the numbers of "active" and "inactive" lever-presses - Attenuation of the reinforcing effects of sucrose



*P<0.05 and **P<0.01 vs. respective vehicle, Newman-Keuls test

Figure 12 Prevention by acute administration of bifeprunox of the cue-induced reinstatement of seeking for sucrose pellets in male Sprague-Dawley rats - Attenuation of the sucrose craving (food craving or psychological dependence)



[a]p<0.05 vs. no reward-associated cue
[b]p<0.05 vs. sucrose-associated cue

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/062555

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/496 A61P25/30
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/130640 A (NUPATHE INC [US]; SEBREE TERRI B [US]; SIEGEL STEVEN J [US]) 30 October 2008 (2008-10-30) claims 1,4,12	1-15
X	WO 2009/090556 A (TASSIN THOMAS [FR]) 23 July 2009 (2009-07-23) page 1; claims 1,2 page 7, line 35 - page 8, line 2	1-15
X	WO 2008/069970 A (ARADIGM CORP [US]; GONDA IGOR [US]) 12 June 2008 (2008-06-12) page 1, paragraph 110; claims	1-15
X	WO 2009/035473 A (SANFILIPPO LOUIS C [US]) 19 March 2009 (2009-03-19) claims 1,12	1-15

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 November 2010	30/11/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Blott, Catherine

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/062555

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARTINOTTI GIOVANNI ET AL: "Efficacy and safety of aripiprazole in alcohol dependence", AMERICAN JOURNAL OF DRUG AND ALCOHOL ABUSE, vol. 33, no. 3, 2007, pages 393-401, XP009126913, ISSN: 0095-2990 * abstract	1-15
X	STOOPS ET AL: "A low dose of aripiprazole attenuates the subject-rated effects of d-amphetamine", DRUG AND ALCOHOL DEPENDENCE, ELSEVIER SCIENTIFIC PUBLISHERS, IR, vol. 84, no. 2, 15 September 2006 (2006-09-15), pages 206-209, XP005585869, ISSN: 0376-8716 * abstract	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 2008069970 A	12-06-2008	EP 2086317 A2	12-08-2009
		EP 2086527 A2	12-08-2009
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