ORIGINAL INVESTIGATION

Attenuation of cocaine and heroin seeking by μ-opioid receptor antagonism

Chiara Giuliano · Trevor W. Robbins · David R. Wille · Edward T. Bullmore · Barry J. Everitt

Abstract

Rationale Evidence has implicated the endogenous opioids, in particular μ-opioid receptors, in emotional behavior and regulation of reward circuits, especially in the context of heroin addiction and hedonic responses to ingestive rewards. The μ-opioid receptor antagonist naltrexone (NTX) has been reported to be effective in preventing relapse to alcoholism and in reducing alcohol and cocaine craving during abstinence.

Objectives The aim of the present experiments was to investigate the effects of a novel selective μ-opioid receptor antagonist GSK1521498 on cocaine and heroin seeking and the primary reinforcement of drug self-administration behavior.

Methods Rats were first trained to self-administer cocaine or heroin and then to seek the drugs over prolonged periods of time under a second-order schedule of reinforcement, in which responding is maintained by contingent presentation of a drug-associated conditioned reinforcer. On a stable baseline, animals were treated with either GSK1521498 (0.1, 1, 3 mg/kg; IP) or NTX (0.1, 1, 3 mg/kg; SC) before each test session.

Results Cocaine seeking was dose-dependently decreased following GSK1521498 treatment. However, the same treatment had no effect on cocaine self-administration under a continuous reinforcement schedule. Treatment with NTX had a less pronounced but similar effect. GSK1521498, but not NTX, dose-dependently reduced heroin seeking both before and after infusion of the drug although both increased heroin self-administration under continuous reinforcement.

Conclusions These data suggest that GSK1521498, by reducing opioid receptor signaling at the μ-opioid receptor, may have therapeutic potential to reduce the propensity to seek cocaine or heroin and, additionally, to diminish the consequence of an initial relapse to heroin taking.

Keywords Addiction · Cocaine seeking · Heroin seeking · Conditioned stimulus · Naltrexone · μ-opioid receptor antagonist

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Introduction

A fundamental feature of addiction to drugs is the propensity to relapse to drug use after short or even prolonged periods of abstinence. Drug-associated environmental stimuli that trigger craving, drug seeking, and relapse are critical factors and provide targets for treatments designed to prevent the tendency to relapse and thereby promote abstinence.

Opioid neurotransmission is implicated in responding and adaptation to emotionally salient stimuli in both animals and humans (Zubieta et al. 2003; Moles et al. 2004). Transmission via μ-opioid receptors mediates an important component of the primary reinforcing effects of opiates such as heroin (Ettenberg et al. 1982) and is also implicated in alcohol (Lê et al. 1999), but not stimulant, drug reinforcement mechanisms (Ettenberg et al. 1982). Activation of the opioid system may also play a role in mediating conditioned incentive effects of stimuli associated with drugs of abuse (Zubieta et al. 2005).

In animal studies, opioid receptor antagonists (such as naltrexone and naloxone) have been shown to reduce cue-elicited reinstatement after extinction of heroin- (Shaham and Stewart 1996), methamphetamine- (Anggadiredja et al. 2004), nicotine- (Liu et al. 2009), ethanol- (Burattini et al. 2006), and cocaine-seeking behavior (Burattini et al. 2008), as well as to reduce alcohol preference in alcohol-dependent rats (Marfaing-Jallat et al. 1983) and drinking in rats selectively bred for high-alcohol preference (Froehlich et al. 1990). Naltrexone has also been reported to reduce rates of relapse to heavy drinking (Volpicelli et al. 1995), providing the basis for approval by the Food and Drug Administration of its use for the treatment of alcohol and opioid dependence.

In the present study, we investigated the effects of GSK1521498, a novel selective μ-opioid receptor antagonist in clinical development for behavioral and drug addictive disorders (Nathan et al. 2011, 2012), in comparison to naltrexone (NTX), on cocaine- and heroin-seeking behavior measured under a second-order schedule of reinforcement, in which prolonged periods of drug-seeking behavior are maintained by contingent presentation of a drug-associated conditioned reinforcer. This procedure allows the effects of the compounds on drug seeking to be measured both before and after cocaine or heroin have been self-administered. We have also assessed the effects of the two compounds on the reinforcing effects of cocaine and heroin in rats self-administering the drugs under a continuous reinforcement schedule. GSK1521498 has been reported to act more selectively than NTX as an antagonist of exogenous agonist activation at the μ-opioid receptor and, under conditions of high receptor expression and endogenous receptor activation in cellular assays, to have inverse agonist properties, thereby reducing receptor activation in the absence of an exogenous agonist. By contrast, NTX has been reported to have partial agonist activity (about 20 % of maximum agonist activity) at μ-opioid receptors (Ignar et al. 2011; Rabiner et al. 2011).

The results of the present study show that reducing opioid receptor signaling at the μ-opioid receptor by GSK1521498 may have therapeutic potential by decreasing drug stimulus-maintained cocaine and, unlike naltrexone, heroin seeking, having the additional effect of diminishing the impact of an acute relapse, or "lapse" to heroin taking, thereby promoting abstinence.

Materials and methods

Animals

Seventy four adult male Lister Hooded rats (Charles River, Margate, UK) weighing 300–320 g at the beginning of the experiments were individually housed under a reversed 12-h light/dark cycle (lights off at 7:00 AM). After a week of habituation to the animal facility where food and water were available ad libitum, rats were maintained on a diet of 20 g of lab chow (Purina) per day given at the end of each daily experimental session. Experiments were performed between 9 AM and 5 PM, 6–7 days a week, and were conducted in accordance with the UK (1986) Animal (Scientific Procedures) Act (Project license 80/2234) and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

Apparatus

Behavioral training was conducted in operant chambers (Med Associates, St. Albans, VT, USA) enclosed within a sound-attenuating box containing a fan to eliminate extraneous background noise. Each chamber was equipped with two retractable levers (4 cm wide, 12 cm apart, and 8 cm from the grid floor), a cue light (2.5 W, 24 V) above each lever, and a white house light (2.5 W, 24 V) at the back of the chamber, in front of the levers. Silastic tubing shielded with a metal spring extended from each animal's IV catheter to a liquid swivel (Stoelting, Wood Dale, IL, USA) mounted on an arm fixed outside of the operant chamber. Tygon tubing extended from the swivel to a Razel infusion pump (Semat Technical, Herts, UK) located adjacent to the external chamber. Lever presses, the light stimulus presentation, the reward delivery, and data collection were controlled by a PC running Whisker control software (Cardinal and Aitken, http://www.whiskercontrol.com).

Surgery

Rats were anesthetized with ketamine hydrochloride (100 mg/kg, IM; Ketaset) and xylazine (9 mg/kg, IM; Rompun) and implanted with a single catheter in the right jugular vein. Catheters were made from 22-gauge stainless steel cannulae attached to Silastic tubing (0.012 ID) and fixed to nylon mesh, which was sutured subcutaneously (SC) between the scapulae. Rats were injected SC from the day before to 7 days post-surgery with the antibiotic 10 mg/kg Baytril (Bayer, Wuppertal, Germany).

Procedure

Daily experimental testing began 7–10 days after intravenous surgery. On each testing day, rats were connected to the IV line before starting the training session. Active and inactive levers were counterbalanced between left and right sides for individual animals. The first step of the training was a fixed ratio 1 (FR1) schedule of reinforcement: rats were trained to self-administer cocaine (0.25 mg/infusion) or heroin (0.04 mg/infusion) by pressing a lever. Each lever

press resulted in illumination of a light stimulus (conditioned stimulus, CS) above the active lever for 20 s, retraction of both levers, and extinction of the house light for 20 s (time out, TO). After this TO, the house light was again illuminated, the CS was extinguished, and the levers were again inserted into the chamber. The responses on the inactive lever had no programmed consequences. Rats were limited to maximum of 30 infusions during each 2-h session. Following the acquisition of cocaine or heroin self-administration (3–5 days), a fixed interval (FI) schedule of reinforcement was introduced as the second step of the training. The FI increased daily from 1 to 2, 4, 8, and 10 min, before stabilizing at FI15 for three consecutive sessions. Subsequently, a second-order schedule of reinforcement was introduced, in which every tenth active lever press resulted in a short 1-s CS presentation [FI15(FR10:S)]. This brief CS presentation is optimal for its effect as a conditioned reinforcer (Everitt and Robbins 2000). At the tenth active lever press after the FI15 had elapsed, cocaine or heroin was infused, and the CS was presented for 20 s, i.e., immediately prior to, during, and immediately after the infusion. Sessions terminated after either five infusions or 2 h, whichever criterion was met first.

Drugs

GSK1521498 was supplied by GlaxoSmithKline (Harlow, UK) in an aqueous stock solution of 4 mg/ml, expressed in terms of the free base, in an acidified hydroxypropyl beta-cyclodextrin-containing vehicle. Prior to administration, the stock solution was diluted with a phosphate buffer to yield 1 or 0.1 mg/ml solutions and filtered through a 0.22-micron filter. The formulation components have been optimized to yield a final solution pH of 5.2–6.5 and osmolality of ~290 mOsm/kg. The vehicle solution, the composition of which was identical to the GSK1521498 stock solution save for omission of the drug substance, was also supplied by GlaxoSmithKline (Harlow, UK) and diluted with phosphate buffer.

NTX (Sigma, UK) was dissolved in sterile physiological saline to yield 3, 1, or 0.1 mg/ml solutions. NTX doses were selected in order to have the greatest selectivity for μ-opioid receptors. At higher doses (i.e., 10 mg/kg), this μ selectivity is lost in favor of increased antagonist potency at δ-opioid receptors (Stromberg et al. 1998). Moreover, the most effective and faster subcutaneous route of administration of NTX was selected in order optimally to compare its effects with those of GSK1521498. NTX injected subcutaneously is 30-fold more potent than when injected intraperitoneally (e.g., in reducing ethanol self-administration in rats) (Williams and Broadbridge 2009). GSK1521498 doses, pre-testing time, and administration route were

based on previous studies (Giuliano et al. 2012; Ignar et al. 2011); the low solubility of the molecule in water resulting in its formulation in a cyclodextrin vehicle makes it unsuitable for subcutaneous injection.

All the solutions were prepared freshly on each test day, and in all studies, the different doses used were administered in a counterbalanced order following a Latin square design. Two or 3 days of washout and re-baselining of behavior were given between each treatment. Independent cohorts of rats were used to study GSK1521498 or NTX effects.

Experiments 1A–1B and 2A–2B: effect of GSK1521498 and NTX on cocaine or heroin seeking

Rats were trained to self-administer cocaine ($n=12$ for GSK1521498 and $n=17$ for NTX) or heroin ($n=19$ for GSK1521498 and $n=11$ for NTX) under a second-order schedule of reinforcement. Following extensive second-order training (>1 month), animals were treated with GSK1521498 (IP) or NTX (SC) 0, 0.1, 1, and 3 mg/kg, 30 and 10 min before test, respectively.

Experiments 3A–3B and 4A–4B: effect of GSK1521498 and NTX on cocaine or heroin self-administration

The same rats trained to self-administer cocaine or heroin under second-order schedule of reinforcement were trained to respond for cocaine under FR1 ($n=8$ for GSK1521498 and $n=14$ for NTX) and FR10 ($n=8$ for GSK1521498 and $n=11$ for NTX) or heroin under FR1 ($n=9$ for GSK1521498 and $n=7$ for NTX) and FR10 ($n=8$ for GSK1521498 and $n=9$ for NTX) schedules of reinforcement. Following stable responding (after 3–6 sessions), animals were treated with GSK1521498 (IP) or NTX (SC) 0, 0.1, 1, and 3 mg/kg, 30 and 10 min, before test, respectively. Rats were limited to a maximum of 30 infusions during each 1-hour session, during both training and test sessions.

Statistical analyses

For the cocaine- and heroin-seeking experiments, active and inactive lever responses during the first (pre-drug infusion) and second (post-drug infusion) 15-min intervals were analyzed using repeated measures analysis of variance (SPSS 19 and SAS 9.2) with dose as the within-subjects factor.

For the cocaine and heroin self-administration experiments, the number of infusions per session was analysed. For all analyses following confirmation of significant main effects, differences between individual treatments compared to control were analysed using the Dunnett's post hoc test. Statistical significance was set at $p<0.05$.

In addition, a second analysis was performed to understand and compare these changes, and investigate differences in ratio of behavioral effects between the GSK1521498- and NTX-treated groups. This random effect analysis, which reported proportional changes, was computed on a log scale.

Results

Experiment 1A: effects of GSK1521498 on cocaine seeking

Twelve rats acquired responding for cocaine under a second-order schedule, making ~300 responses during the first, drug-free 15-min interval. One animal was excluded from the analysis due to ill health. Treatment with GSK1521498 resulted in a significant dose-dependent decrease in responding during the first (pre-cocaine infusion) 15-min interval of the session [$F(3,30)=8.9$; $p<0.001$]. This effect was specific to the active lever since inactive lever responding was unaffected [$F<1$]. However, GSK1521498 had no effect on active lever presses during the second interval (post-cocaine infusion) [$F<1$]. Post hoc analysis revealed a significant effect of GSK1521498 0.1, 1, and 3 mg/kg during the first interval of the session ($p<0.05$ for each dose) (Fig. 1).

Experiment 1B: effects of NTX on cocaine seeking

Twenty animals acquired lever pressing for cocaine under the second-order schedule making ~300 responses in 15 min for the first cocaine infusion. Three animals were excluded from further analysis because of catheter failure. Treatment with NTX resulted in a significant dose-dependent decrease in seeking responses during the first (pre-cocaine infusion) 15-min interval of the session [$F(3,48)=11.2$; $p<0.001$] but had no effect in the second interval after cocaine had been self-administered [$F<1$]. Post hoc analysis revealed the first interval decrease in responding to be significant at 0.1, 1, and 3 mg/kg ($p<0.01$ for each dose). There were no significant effects of NTX on inactive lever responding [first and second intervals: $F<1$] (Fig. 2).

### Comparison between GSK1521498 and NTX effects on cocaine seeking

Comparing the relative effect of GSK1521498 over NTX on cocaine seeking, there was a statistically significant, 2.55-fold greater reduction of active lever pressing in the first 15-min interval following GSK1521498 3 mg/kg compared to NTX 3 mg/kg ($p=0.041$). Similar but statistically non-significant effects were seen at lower doses: 1.75- and 1.42-fold greater reductions of active lever pressing following GSK1521498 compared to NTX at 1 and 0.1 mg/kg, respectively. A graphical summary of these results, and of the other GSK1521498 to NTX ratio comparisons, is represented as 95 % confidence intervals in Fig. 6.

### Experiment 2A: effects of GSK1521498 on heroin seeking

Twenty rats acquired responding for heroin under a second-order schedule, making ~250 responses during the 15 min before the first heroin infusion. One animal was excluded from the analysis due to catheter failure.

Treatment with GSK1521498 resulted in a significant, dose-dependent decrease in responding during the first (pre-heroin infusion) 15-min interval of the session [F

Fig. 1 Effects of GSK1521498 on cocaine seeking under a second-order schedule of reinforcement during the first drug-free interval (**a**, **b**) and after cocaine infusion (second interval) (**c**, **d**). Data shown are mean (+SEM) number of presses on the active (**a**, **c**) and inactive lever (**b**, **d**). *Single asterisk* (*) $p<.05$ compared with vehicle treated animals
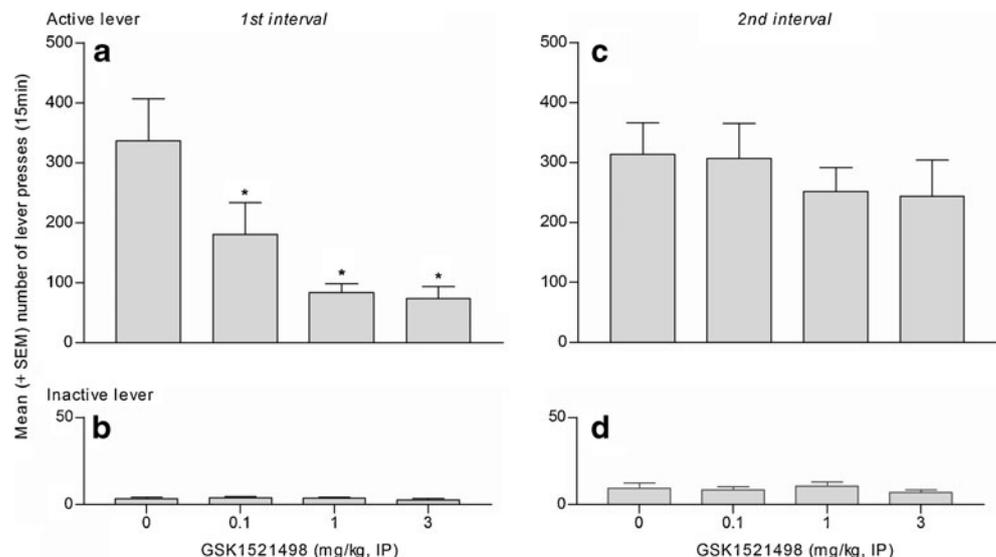
Fig. 2 Effects of naltrexone on cocaine seeking under a second-order schedule of reinforcement during the first drug-free interval (**a**, **b**) and after cocaine infusion (second interval) (**c**, **d**). Data shown are mean (+SEM) number of presses on the active (**a**, **c**) and inactive lever (**b**, **d**). *Double asterisk* (******) *p*<.01 compared with vehicle treated animals
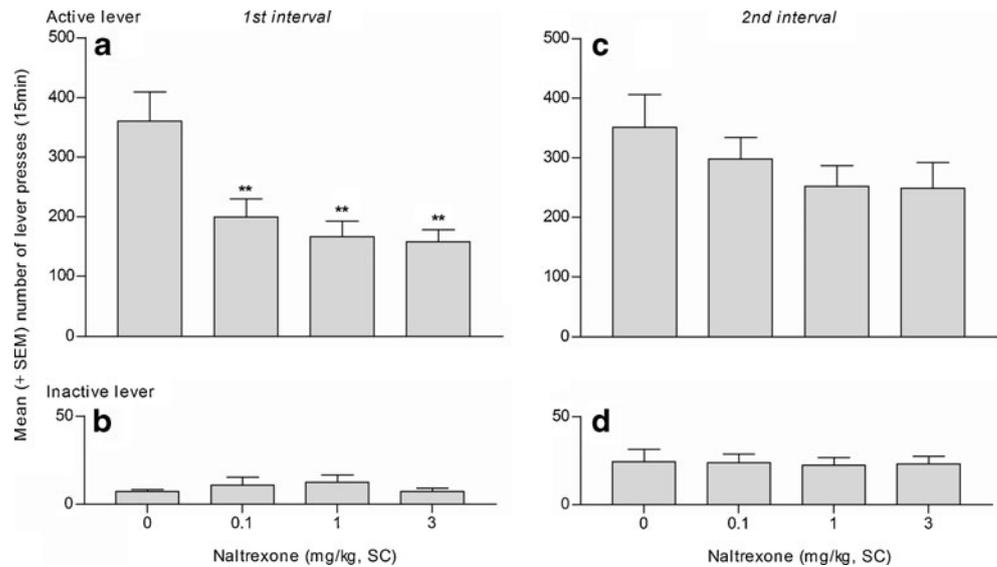
(3,54)=18.82; *p*<0.001], an effect that was specific to the active lever since inactive lever responding was unaffected [*F*<1]. GSK1521498 also reduced responding during the second interval (post-heroin infusion) [*F* (3,54)=12.44; p<0.001]. Post hoc analysis revealed significant decreases in first interval responding at the 0.1 (*p*<0.01), 1 (*p*<0.001), and 3 mg/kg (*p*<0.001) doses of GSK1521498, an increase in second interval responding at 0.1 mg/kg (*p*<0.01) and a decrease in second interval responding at 3 mg/kg (*p*<0.05) doses (Fig. 3).

Experiment 2B: effects of NTX on heroin seeking

Eleven rats acquired responding for heroin under a second-order schedule, making ~290 responses in

15 min for the first infusion of heroin. Treatment with NTX had no effect on responding during the first [*F*<1] or the second [*F*<1] intervals, i.e., neither before nor after heroin infusion (Fig. 4).

Comparison between GSK1521498 and NTX effects on heroin seeking

Compared to NTX, GSK1521498 caused a greater reduction in response under all testing conditions except following the lowest dose in the second interval. Furthermore, for all but the lowest dose, these effects were statistically significant at the 5 % level. Dose-dependent fold change reductions in heroin-seeking behavior following GSK1521498 compared to NTX were estimated as 1.66 (0.1 mg/kg), 4.53 (1 mg/kg),

Fig. 3 Effects of GSK1521498 on heroin seeking under a second-order schedule of reinforcement during the first drug-free interval (**a**, **b**) and after heroin infusion (second interval) (**c**, **d**). Data shown are mean (±SEM) number of presses on the active (**a**, **c**) and inactive lever (**b**, **d**). *Single asterisk* (***) *p*<.05, *double asterisk* (****) *p*<.01, and *triple asterisk* (*****) *p*<.001 compared with vehicle treated animals
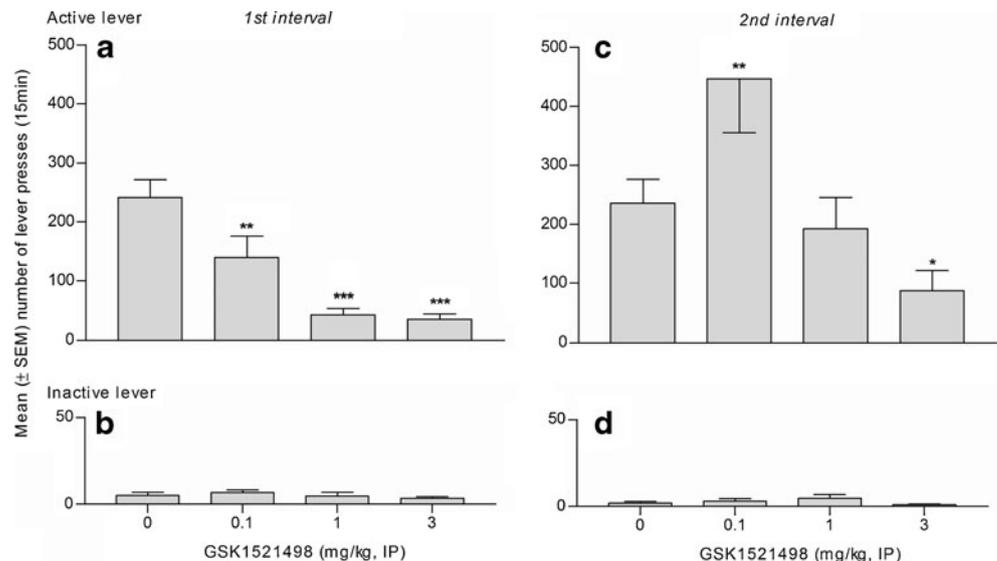
Fig. 4 Effects of naltrexone on heroin seeking under a second-order schedule of reinforcement during the first drug-free interval (**a**, **b**) and after heroin infusion (second interval) (**c**, **d**). Data shown are mean (+SEM) number of presses on the active (**a**, **c**) and inactive lever (**b**, **d**)
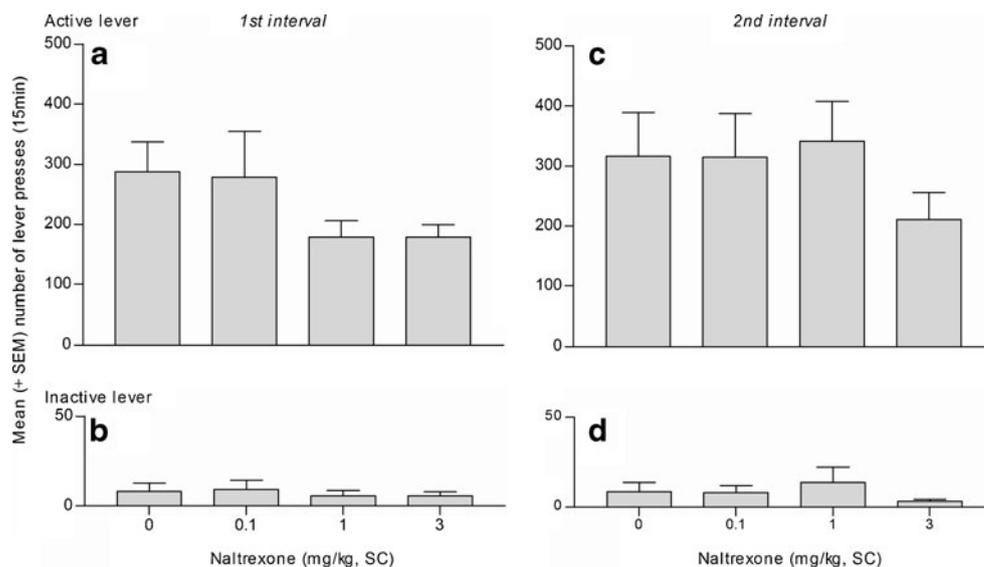
and 5.50 (3 mg/kg) for the first interval and 2.43 (1 mg/kg) and 3.02 (3 mg/kg) for the second interval (Fig. 6).

Experiments 3A–3B: effects of GSK1521498 and NTX on cocaine self-administration

GSK1521498 and NTX had no effect on cocaine self-administration under either FR1 or FR10 schedules of reinforcement [$F<1$] (Fig. 5).

Experiments 4A–4B: effects of GSK1521498 and NTX on heroin self-administration

GSK1521498 0.1, 1, and 3 mg/kg increased the number of heroin infusions [$F(3,24)=9.9$; $p=0.001$] under FR1 schedule of reinforcement ($p<0.01$ for each dose). When the schedule of reinforcement was increased to FR10, GSK1521498 1 mg/kg similarly increased the number of infusions earned per session ($p<0.001$) (Fig. 5).

Treatment with NTX 0.1, 1, and 3 mg/kg resulted in an increased number of heroin infusions [$F(3,18)=24.4$; $p<0.001$] under a FR1 schedule of reinforcement ($p<0.001$ for each dose). When the schedule of reinforcement was increased to FR10, NTX 1 mg/kg similarly increased the number of infusions earned per session ($p<0.001$) (Fig. 5).

There were no significant differences between GSK1521498 and NTX in their effects on heroin or cocaine self-administration (Fig. 6).

Discussion

The present study shows that selective antagonism at the μ-opioid receptor by the novel compound GSK1521498

markedly reduced both cocaine and heroin seeking in comparison to NTX, which had no effect on heroin seeking and a significantly lesser effect on cocaine seeking. In addition, GSK1521498 also decreased heroin, but not cocaine, seeking after the first drug infusion had been self-administered. Neither GSK1521498 nor NTX had any effect on the self-administration of cocaine, i.e., they did not alter the primary reinforcing effects of cocaine but did, as expected, result in an increase in the self-administration of heroin, confirming results of earlier studies (Ettenberg et al. 1982).

Opioid neurotransmission, in particular via μ-opioid receptors, is implicated in regulating food intake and in mediating the rewarding impact of palatable food (Nathan and Bullmore 2009), but it has also been suggested to impact incentive salience attribution to CSs that predict reward (Peciña 2008), and μ-opioid receptor knockout mice show a reduced food-anticipatory activity (Kas et al. 2004). Moreover, μ-opioid receptor agonists infused into the NAcc increase the motivation to work for highly palatable food prior to its delivery (Zhang et al. 2003). Previously, we have shown using a second-order schedule of highly palatable food (chocolate) reinforcement that GSK1521498, but not NTX, significantly reduced chocolate seeking as well as binge eating (Giuliano et al. 2012). In the context of (1) the clinical use of NTX to decrease alcohol (Volpicelli et al. 1992) and cocaine (Schmitz et al. 2001) craving and (2) findings suggesting that opioid receptor signaling mediates conditioned effects of environmental stimuli associated with drugs of abuse (Zubieta et al. 2005), we investigated in detail the effect of GSK1521498 and NTX under conditions in which drug-associated incentive stimuli are critically important for maintaining heroin- and cocaine-seeking behavior over extended delays to reinforcement.
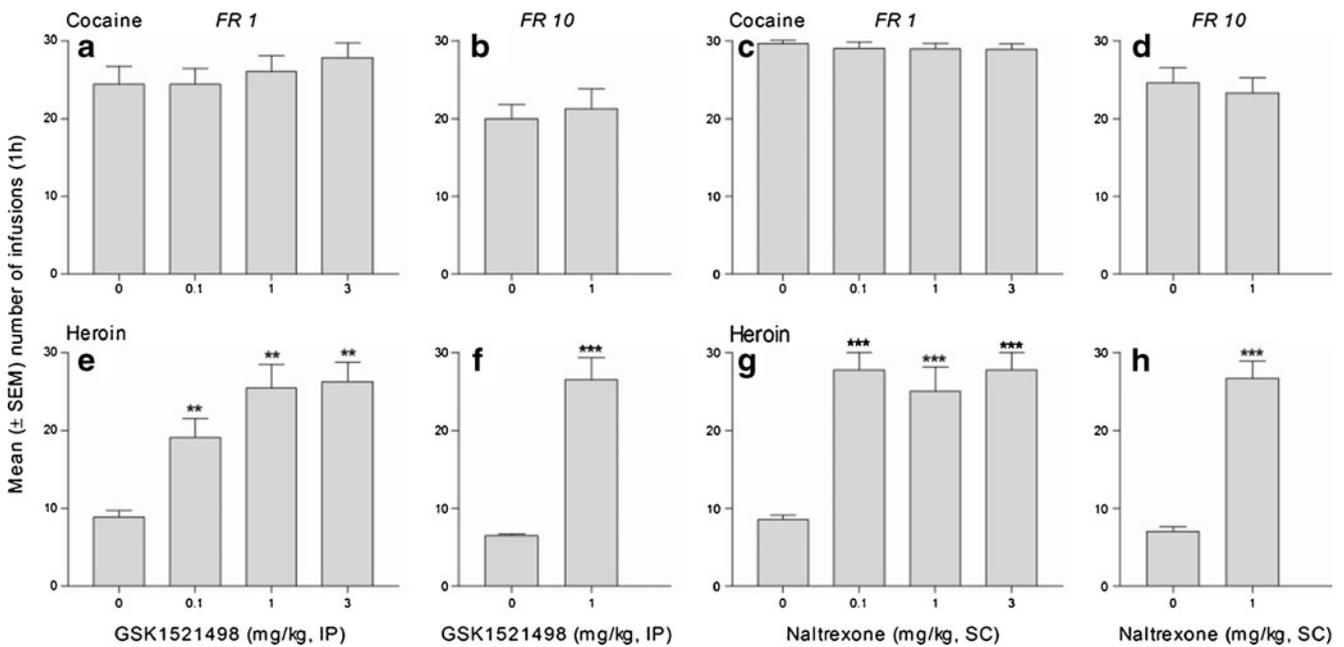
Fig. 5 Top: effects of GSK1521498 (**a**, **b**) and naltrexone (**b**, **c**) on cocaine self-administration under a fixed-ratio 1 (**a**, **c**) and a fixed-ratio 10 (**b**, **d**) schedule of reinforcement. Bottom: effects of GSK1521498 (**e**, **f**) and naltrexone (**g**, **h**) on heroin self-administration under a fixed- ratio 1 (**e**, **g**) and a fixed-ratio 10 (**f**, **h**) schedule of reinforcement. Data shown are mean (+SEM) number of infusions reached per session. *Double asterisk* (******) $p < .01$ and *triple asterisk* (*******) $p < .001$ compared with vehicle treated animals

Heroin seeking

The present results confirmed those of our initial study that also failed to show an effect of naloxone on heroin seeking

(Alderson et al. 2000). However, by the third day of naloxone treatment, as the rats presumably learned about the decreased value of heroin under naloxone (Alderson et al. 2000), responding for heroin decreased, leading to the
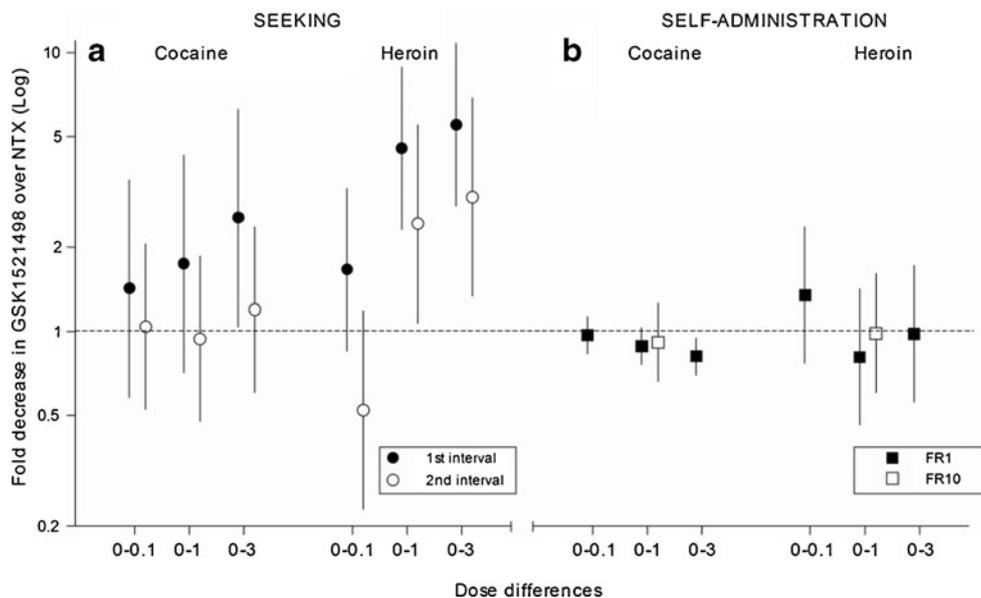
Fig. 6 Differential efficacy of GSK1521498 compared to naltrexone on cocaine and heroin seeking (**a**) and self-administration behavior (**b**). Each *vertical line* in the plot is a 95 % confidence interval for the fold difference in effect of GSK1521498 versus NTX at each of three doses (0.1, 1, and 3 mg/kg) in log scale. *Positive values* indicate that GSK1521498 has a greater effect than NTX, causing greater reduction or fold decrease in cocaine- or heroin-seeking behavior; the *horizontal line* highlights a fold difference of 1 or equivalent efficacy of the two drugs. If a confidence interval does not include 1, that is equivalent to a statistically significant difference in efficacy of GSK1521498 compared to NTX

gradual extinction of heroin-seeking behavior. In the present study, under identical schedule conditions and acute treatment, GSK1521498, but not NTX, markedly reduced responding during the first interval of the session and, at the highest dose, also after the first heroin infusion was earned. This result suggests that selective inhibition at μ-opioid receptors is required to reduce cue-induced heroin seeking, and this does not simply depend on a devaluation of the effects of heroin by the opiate receptor antagonist. In addition, GSK1521498 also affected seeking behavior, as measured by active lever responses after heroin had been self-administered, increasing responding at the lowest dose and markedly decreasing it at the highest dose. This post-heroin infusion inverted U-shaped dose–response function likely reflects, successively, the reduced and then more completely antagonized reinforcing effects of the drug by GSK1521498. When GSK1521498 and NTX were given prior to a heroin self-administration session under the less demanding continuous or FR10 reinforcement schedules, both as expected increased the number of infusions in 1-hour sessions in a dose-dependent manner, i.e., the animals responded to increasing doses of the antagonist by increasing their intake of heroin (Ettenberg et al. 1982). However, the maximum number of 30 infusions allowed in each 1-hour session may have precluded a clear dose–response distinction between the doses of 1 and 3 mg/kg, for both GSK1521498 and NTX. Previous studies have clearly demonstrated the effect of inhibiting opioid receptor signaling to reduce heroin-induced (Shaham and Stewart 1996) and intra-VTA morphine-induced (Stewart 1984) reinstatement of responding after extinction, as well as to reduce responding to heroin-paired cues (Sorge et al. 2005). However, these procedures, based on instrumental extinction and reinstatement, do not readily allow distinctions to be made between the effects of μ-opioid receptor antagonists on extinction learning (Peters et al. 2008) versus cued mechanisms of relapse. The present data strongly indicate an effect on the conditioned control over heroin seeking.

Cocaine seeking

The results described in the present study further showed that GSK1521498 reduced cocaine seeking and that it did so significantly more effectively than NTX. The effects of both compounds were in this case specific to cocaine seeking since there was no effect on responding after the cocaine infusion had been delivered. Moreover, neither GSK1521498 nor NTX altered the self-administration of cocaine under a simple fixed ratio schedule, indicating modulation of mechanisms regulating cocaine-seeking behavior rather than cocaine reinforcement. Previous studies have suggested that the mixed μ-opioid receptor agonist–antagonist buprenorphine and NTX might be useful in treating cocaine

dependence. Buprenorphine has been reported effectively to reduce cocaine and opioid self-administration in both humans (Kosten et al. 1989) and laboratory animals (Mello et al. 1990; Carroll and Lac 1992), and NTX was also effective in suppressing cocaine and heroin self-administration in humans (Kosten et al. 1989) and monkeys (Mello et al. 1990). However, the effects of NTX in rats are less consistent, having been reported to increase (Carroll et al. 1986), to decrease (Corrigall and Coen 1991), or, as reported here, to have no effect on cocaine self-administration (Ettenberg et al. 1982). Acute NTX treatment also had no effect on cocaine-induced reinstatement of responding (Comer et al. 1993), whereas the sub-chronic pre-treatment progressively attenuated cocaine-induced reinstatement of extinguished responding (Gerrits et al. 2005). NTX has been reported to diminish cocaine seeking induced by re-exposure to cocaine-associated cues after extinction (Burattini et al. 2008), an effect consistent with that reported here, that was itself less pronounced than the effect of GSK1521498.

The apparently greater efficacy of GSK1521498 compared to NTX in reducing cocaine, and especially heroin, seeking may reflect different opioid receptor subtype selectivities. GSK1521498 is 14- and 20-fold selective for human μ- over κ- and δ-opioid receptors and is 52- and 66-fold selective for rat μ- over κ- and δ-opioid receptors, whereas NTX is 4- and 10-fold selective at human μ- versus κ- and δ-opioid receptors and 20- and 25-fold selective at rat μ-compared with κ- and δ-opioid receptors (Ignar et al. 2011). The mean exposures achieved following a 3-mg/kg dose of GSK1521498 in mice and rats are broadly similar: AUC $(0–\infty)$ and Cmax of 5,254.6 and 887.9 ng/ml for mice, compared to 9,159.1 and 1,082.7 ng.h/ml for rats. Thus, it seems reasonable to assume that equivalent doses of GSK1521498 translate into approximately equivalent plasma concentrations in both rat and mouse. Based on the μ-opioid receptor occupancy of GSK1521498 and NTX measured in mice, GSK1521498 and NTX dose-dependently and completely occupied brain μ-opioid receptors in adult male C57BL/6J mice. The dose achieving 50 % occupancy (OD_{50}) in mice ranged from 0.041 to 0.064 mg/kg for NTX (SC administration). The OD_{50} for GSK1521498 ranged from 1.24 to 2.23 mg/kg (IP administration). Consistent with estimated OD_{50} values, NTX achieved greater MOR occupancy at all dose levels than that achieved by GSK1521498. In mice, equivalent doses up to 3 mg/kg NTX (SC) or GSK1521498 (IP) achieved receptor occupancy of 93–94 % for NTX and 64–73 % for GSK1521498 in the nucleus accumbens and caudate putamen, respectively. While obvious caveats apply in translating μ-opioid receptor occupancy data from mouse to rat, they indicate that NTX achieves greater receptor occupancy than GSK1521498 at equivalent doses. The superior efficacy of GSK1521498 demonstrated behaviorally by these

experiments is therefore not readily attributable to its greater receptor occupancy compared to NTX at equivalent doses. A more likely explanation follows from the prior observation that NTX has partial agonist activity (about 20 % of maximum agonist activity) at the μ-opioid receptor, whereas GSK1521498 has no partial agonist activity and is therefore more completely effective in blocking activation of the receptor by an agonist in cellular assays (Ignar et al. 2011). Thus, a given dose of GSK1521498 putatively has greater effect on μ-opioid receptor agonist-driven behavior than an equivalent dose of NTX not because it achieves greater receptor occupancy but because it has greater net antagonistic activity at the receptor.

Neurobiological mechanisms of cocaine and heroin seeking

The common effect of GSK1521498 to reduce cocaine and heroin seeking, as well as the seeking of highly palatable food (Giuliano et al. 2012), may reflect actions on a common neural mechanism for incentive motivation. Acquisition and/or performance of drug seeking under second-order schedules of reinforcement or the reinstatement of seeking responses depend upon the nucleus accumbens core and its afferents from the basolateral amygdala (Vanderschuren et al. 2005), as well as dopaminergic activity in the dorsal striatum, when previously well established (Belin and Everitt 2008). Drug-seeking behavior is potentiated by drug-induced increases in DA release (Arroyo et al. 1998) and can be reduced by GABAergic inhibition of VTA dopaminergic neurons (Di Ciano and Everitt 2004). One possible locus of the effect of GSK1521498 and NTX to reduce heroin and cocaine seeking may be via blockade of μ-opioid receptors on VTA GABAergic interneurons, thereby increasing GABAergic and decreasing mesolimbic DAergic transmission. Actions within the nucleus accumbens (Di Ciano et al. 2003) or globus pallidus (Smith et al. 2009) to reduce the incentive value of reward-associated CSs (Flagel et al. 2011), each of which can modulate incentive or hedonic motivational responses, are alternative or additional sites of action. The marked effect of GSK1521498 and NTX to reduce cocaine and heroin seeking under second-order schedules of drug reinforcement suggests diminished control over the high levels of responding maintained by drug-associated conditioned stimuli.

Clinical implications

Although NTX is in use clinically both to reduce alcohol craving (Volpicelli et al. 1992) and alcohol intake (Davidson et al. 1999) and in exploratory clinical studies to reduce amphetamine craving (Jayaram-Lindström et al. 2008), the considerable individual differences in response to the drug, perhaps due to its short half-life and mixed antagonist and partial agonist profile, can result in poor medication compliance (McCaul et al. 2000). Moreover, NTX undergoes first-pass metabolism in the liver to form its major, but less active, metabolite 6-β-naltrexol, high urinary levels of which have been found in subjects who experience adverse side effects (King et al. 1997). Although some of the disadvantages of NTX, especially its short half-life, may be avoided using an extended-release injectable form (Vivitrol®), other unwanted effects, such as adverse injection site reactions, have been reported, while its partial agonist effects remain.

Treatment of volunteers with GSK1521498 has demonstrated a longer plasma half-life of the drug and much less first-pass metabolism than NTX. Additionally, GSK1521498 significantly attenuated activation of amygdala and ventral striatum by food-associated stimuli in a functional imaging study, whereas NTX did not (Rabiner et al. 2011). Taken together with the results reported here, we suggest that GSK1521498, with its full μ-opioid receptor antagonist profile, may be more effective therapeutically than NTX, especially in the promotion of abstinence and in the prevention of cocaine and heroin relapse in addicted individuals seeking treatment. Moreover, its unique effect to reduce heroin seeking even after self-administration of an infusion of heroin may indicate its potential use to prevent full or sustained relapse after an initial lapse to heroin taking. These and other behavioral data on binge eating and food seeking (Giuliano et al. 2012) suggest that GSK1521498 can generally attenuate reward-driven or reward-seeking behaviors, whether these are incentivised by chocolate, cocaine, or heroin. This cross-cutting efficacy on multiple species of reward-seeking behavior in animal models suggests that GSK1521498 could have therapeutic efficacy in several clinical disorders characterized by compulsive consumption of rewards. It also potentially raises a risk for clinical development, namely that attenuation of abnormal, compulsive reward-seeking behavior might also attenuate the capacity for more normal responsivity to rewarding stimuli. However, recent clinical data from a study of GSK1521498 in patients with binge eating and obesity, treated for 28 days at doses of 2 and 5 mg/day, demonstrated no evidence for adverse effects of GSK1521498 on measures of mood or the capacity for pleasurable responses to sexual or other normal rewards (Ziauddeen et al. 2012).

These therapeutic predictions, based largely on the results of our pre-clinical behavioral experiments, merit further investigation in translational studies of GSK1521498 for the treatment of patients with substance dependence disorders.

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