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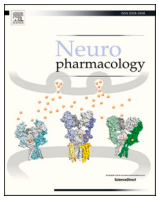

Preclinical evidence for the use of the atypical antipsychotic, brexpiprazole, for opioid use disorder

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A B S T R A C T

Opioid addiction is characterized by adaptations in the mesolimbic dopamine system that occur during chronic opioid use. Alterations in dopaminergic transmission contribute to pathological drug-seeking behavior and other symptoms associated with opioid withdrawal following drug discontinuation, making drug abstinence challenging and contributing to high rates of relapse among those suffering from substance use disorder. Recently, the use of dopamine partial agonists has been proposed as a potential strategy to restore dopaminergic signalling during drug withdrawal, while avoiding the adverse side effects associated with stronger modulators of dopaminergic transmission. We investigated the effects of the atypical antipsychotic brexpiprazole, which is a partial agonist at dopamine D2 and D3 receptors, in a mouse model of opioid dependence. The development of opioid dependence in mice is characterized by locomotor sensitization, analgesic tolerance, opioid-induced hyperalgesia, and drug-seeking behavior. We set up four paradigms to model the effects of brexpiprazole on each of these adaptations that occur during chronic opioid use in male and female C57BL/6J mice. Concomitant treatment of brexpiprazole during chronic morphine administration attenuated the development of locomotor sensitization. Brexpiprazole treatment abolished morphine place preference and blocked reinstatement of this behavior following extinction. Brexpiprazole treatment did not alter morphine analgesia, nor did it impact the development of morphine tolerance. However, brexpiprazole treatment did prevent the expression of opioid-induced hyperalgesia in a tail-withdrawal assay, while failing to improve somatic withdrawal symptoms. Altogether, these results provide preclinical evidence for the efficacy of brexpiprazole as a modulator of dopamine-dependent behaviors during opioid use and withdrawal.

1. Introduction

Opioid use disorder is a chronic relapsing disease, affecting 26.8 million people worldwide and representing a significant individual and public health issue (Strang et al., 2020). Current therapeutics for opioid use disorder are often limited in efficacy, in part due to the difficulty of targeting the profound alterations in central reward circuitry which underlie the pathophysiology of opioid dependence and withdrawal. The majority of drugs of abuse, including opioids, converge on the mesolimbic dopamine system, acutely driving an increase in dopamine in the nucleus accumbens and chronically inducing significant maladaptations in dopaminergic transmission (Nestler, 2005; Koob and Volkow, 2010). These maladaptations have been implicated in the development of pathological drug craving and relapse following drug abstinence. Shared alterations in dopamine signalling by drugs of abuse are reflected by a set of conserved addiction-related behaviors, namely behavioral sensitization to the drug effects (Robinson and Berridge, 2001, 2008), a negative affective and sensory state during withdrawal, and associative learning based on drug cues (Nestler, 2005). Behavioral sensitization is believed to be driven by elevations in striatal dopamine levels during repeat drug administration, which then sensitizes the striatal response to the drug and drug-related cues, driving a progressive increase in drug salience and "wanting" (Robinson and Berridge, 2008; Berridge, 2007; de Vries et al., 1998). Conversely, a decline in extracellular dopamine levels during drug withdrawal following chronic use has been implicated in the development of a negative emotional and motivational state (Koob and Volkow, 2010; Koob, 2020).

Recently, the use of dopamine partial agonists has been proposed as a potential strategy to restore normal dopaminergic tone during the hypodopaminergic withdrawal state, while avoiding the adverse side effects associated with stronger modulators of dopaminergic transmission (Moreira and Dalley, 2015; Das et al., 2016). Partial agonists can behave both as functional antagonists in the presence of a full agonist and functional agonists in the absence of alternative receptor ligands. A partial dopamine agonist is therefore able to modulate the effect of dopamine (the endogenous receptor ligand, which acts as a full agonist) at its receptors, attenuating receptor activation when dopamine levels are high (i.e. following drug administration) and increasing it when

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dopamine levels are low (i.e. during drug withdrawal). Because these agonists will only partially activate the receptor even at maximal doses, side effects associated with excessive dopamine activation are reduced. Dopamine partial agonism underlies the mechanism of multiple atypical antipsychotics, where these drugs are used clinically to stabilize the mesolimbic dopamine system during psychosis (Frampton, 2019). Consequently, atypical antipsychotics acting via dopamine partial agonism may also lend themselves to the regulation of the dysfunctional reward circuitry seen in drug dependence and addiction.

Brexpiprazole is an atypical antipsychotic that, like its predecessor aripiprazole, acts as a dopamine-system stabilizer (Maeda et al., 2014a, 2014b; Kikuchi et al., 2021). Currently approved for the treatment of both schizophrenia and major depressive disorder (McKeage, 2016), both brexpiprazole and aripiprazole act primarily as partial agonists at D2/D3 receptors and 5-HT1A receptors, while exerting antagonistic effects at 5-HT2A receptors. While aripiprazole and brexpiprazole exhibit similar affinities at dopamine receptors (Maeda et al., 2014a, 2014b), brexpiprazole exhibits higher affinity for 5-HT1A/2A, lower intrinsic activity at D2 receptors, and overall appears to be well tolerated at clinical doses (Das et al., 2016; Kane et al., 2016). Given brexpiprazole's lower intrinsic activity at dopamine receptors compared to aripiprazole, it has lower potential for eliciting the dopamine-related side effects commonly associated with antipsychotics (McKeage, 2016; Amada et al., 2019).

Several studies have investigated aripiprazole as a potential dopamine system stabilizer in preclinical models of addiction, demonstrating its ability to attenuate sensitization to the behavioral effects of drugs of abuse (Leite et al., 2008; Narita et al., 2008; Almeida-Santos et al., 2014), and block drug-paired chamber preference and reinstatement of drug-seeking behavior in a conditioned place preference (CPP) paradigm (Narita et al., 2008; Almeida-Santos et al., 2014; xia Li et al., 2009). Aripiprazole, in addition to modulating dopamine-associated behaviors, also has been shown to lower morphine-induced increases in dopamine levels within the nucleus accumbens (Narita et al., 2008). Recently, several studies have investigated the use of aripiprazole in substance use disorder populations (Meini et al., 2011; Szerman et al., 2020; Coles et al., 2021; Martinotti et al., 2022), with aripiprazole showing either no or slight improvement in outcomes related to substance use. However, most of these studies involved comorbid substance use and psychosis, and none specifically examined the effects of aripiprazole on opioid use disorder.

Given the promising results seen in opioid dependence and addiction models with aripiprazole, we aimed to investigate the effects of brexpiprazole as a potential partial agonist therapy in a mouse model of opioid dependence. To do so, we assessed the ability of brexpiprazole to modulate the behavioral responses associated with drug-induced adaptations during chronic opioid administration and withdrawal.

2. Methods

2.1. Animals

Male and female C57BL/6J mice aged 9–12 weeks at the beginning of each experiment were received from Jackson Laboratories (Bar Harbor, ME). All animals were housed in wire-top cages (4–5 per cage) in a temperature- and humidity-controlled environment with free access to water and food. Mice were kept on a 12 h:12 h dark/light cycle, with all behavioral experiments performed during the light cycle. Prior to all experiments, mice were habituated to the novel behavior testing room and handled. All animal experiments and procedures were conducted in compliance with the Canadian Council on Animal Care Guidelines and Policies with approval from the University of Alberta Health Sciences Animal Care and Use Committee.

2.2. Drugs

Morphine sulfate pentahydrate powder was purchased from Toronto Research Chemicals (Toronto, Ontario) and dissolved in 0.9% w/v NaCl. Brexpiprazole was purchased in powder form from Cayman Chemical (Ann Arbor, Michigan) and dissolved in either 2.6% or 3.3% DMSO in 0.9% NaCl. All drugs were administered through either a subcutaneous (s.c.) or intraperitoneal (i.p.) route at a volume of 10 mL/kg. The 0.1 mg/kg dose of brexpiprazole used in all experiments was based on previous studies of both brexpiprazole (Milienne-Petiot et al., 2017; Ma et al., 2016) and aripiprazole (Almeida-Santos et al., 2014; xia Li et al., 2009).

2.3. Morphine-induced locomotor sensitization

Locomotor sensitization was achieved using a protocol based on previous reports (Contet et al., 2008). All animals were habituated to the open field arena apparatus prior to the start of the experiment. Male mice were treated once every 72 h over the course of 13 days with s.c. injections of either brexpiprazole (0.1 mg/kg) or vehicle (3.3% DMSO in saline), followed by an i.p. injection of either morphine (40 mg/kg) or saline (0.9% NaCl) 30 min later. Directly after administration or either morphine or saline, mice were placed in a circular open field arena (45.7 cm diameter) and video tracking software (Noldus Ethovision, Leesburg, VA) was used to measure the total distance travelled within the arena during a 30-min recording period. Mice underwent 5 total sessions over 13 days during which locomotor activity was recorded.

2.4. Morphine-conditioned place preference (CPP)

Morphine CPP was performed using a 2-chamber, counter-balanced, and unbiased apparatus. Each conditioning chamber (24 × 24.5 × 28 cm) was visually distinguishable by a wall pattern of either stripes or spots, and chambers were separated by a removable guillotine door. The time spent in each chamber was recorded using video tracking software (Noldus Ethovision, Leesburg, VA) during the pre-conditioning, post-conditioning, and reinstatement tests, as well as the extinction phase. To control for inherent bias for either chamber, mice received a habituation period to freely explore both chambers for 20 min before a pre-conditioning test on the following day. During the pre-conditioning test, the animals were again allowed to freely explore both chambers for 30 min, and animals were assigned their respective saline- and morphine-paired chambers such that any inherent bias for one chamber in the pre-conditioning test was balanced among vehicle and brexpiprazole treatment groups. Mice were dropped from the study if they spent ≥75% of their pre-conditioning test time in any one chamber, leading to the exclusion of one animal from the brexpiprazole group in this experiment.

2.4.1. Conditioning and post-conditioning tests

Male mice received 8 daily conditioning sessions, during which they received i.p. injections of either saline (0.9% NaCl) or morphine (10 mg/kg) on alternating days, before being confined to either the assigned saline- or morphine-paired chamber for 30 min. Half of the mice in each treatment group received saline injections during the first conditioning session, while the other half received morphine. The day following the final conditioning session, all mice underwent a state-independent post-conditioning test, where they received either brexpiprazole (0.1 mg/kg, s.c.) or vehicle (3.3% DMSO, s.c.) 30 min prior to being placed in the CPP apparatus with free access to both chambers during the 30-min recording period. On the second day after the final conditioning session, mice underwent a state-dependent post-conditioning test, which was identical to the state-independent test with the exception of a priming dose of morphine (10 mg/kg, i.p.) which was administered 30 min after vehicle or brexpiprazole injections and directly prior to being placed in the CPP apparatus.

2.4.2. Extinction and reinstatement

Following both the post-conditioning tests, mice were subjected to an extinction phase which consisted of 4 daily sessions where no drug was administered and free access to both chambers was allowed for 30 min, during which recording was done to track the extinction of preference for the morphine-paired chamber. The day following extinction training, a reinstatement test was performed where all animals received either brexpiprazole (0.1 mg/kg, s.c.) or vehicle (3.3% DMSO, s.c.), and 30 min later received a priming dose of morphine (10 mg/kg, i.p.). Immediately following the priming dose, mice were placed into the CPP apparatus with free access to both chambers and recorded for a 30-min period. For all CPP recordings, data is represented as the time spent in either the saline- or morphine-paired chamber, or the change in morphine-paired chamber (drug chamber) time from pre-to post-conditioning.

2.5. Thermal tail-withdrawal assay

Mice were habituated to the tail-withdrawal apparatus and restraint prior to the experiment. On test days, mice were gently restrained, and approximately 2.5 cm of the distal portion of the tail was immersed in water at 49 °C. The length of time the animal took to withdraw its tail after tail submersion was recorded using a stopwatch. A cut-off latency of 15 s was established to prevent potential tissue damage. For assessing morphine analgesia, a single tail withdrawal baseline was taken per animal post-morphine. For assessing the development of opioid-induced hyperalgesia, 3 baseline measurements were taken for each animal per test, and withdrawal latencies across all three baselines were averaged. Pre-experiment baselines were taken for all animals in the drug-naive state prior to chronic escalating morphine treatment to establish baseline pain thresholds. Tail-withdrawal latencies taken following chronic morphine or saline treatment are represented as the change from baseline in each animal.

2.6. Morphine-induced analgesic tolerance

To establish tolerance to morphine-induced analgesia, twice-daily (9:00 and 17:00) i.p. injections of escalating doses of morphine (10, 20, 30 mg/kg) or saline (0.9% NaCl) were given over 3 days. Male mice received s.c. injections of either brexpiprazole (0.1 mg/kg) or vehicle (3.3% DMSO) 30 min before the morning injection of morphine. Analgesia was monitored using the tail-withdrawal test, 20 min after the morning injection. On the fourth day, mice in all groups received either s.c. brexpiprazole or vehicle, followed by a single test dose of morphine (10 mg/kg, i.p.) 30 min later. A tail-withdrawal test was performed 20 min later and withdrawal latencies for all groups were recorded.

2.7. Establishment of opioid dependence and hyperalgesia

Male and female mice were made opioid-dependent using a protocol consisting of twice-daily (9:00 and 17:00) injections of escalating doses of morphine (10, 20, 30, 40 mg/kg, i.p.) for 4 days. Drug-naïve control animals received twice-daily saline (0.9% NaCl, i.p.) in lieu of drug. Vehicle or brexpiprazole (0.1 mg/kg, s.c.) was given 30 min prior to the morning dose of either saline or morphine. All behavioral tests in opioid-dependent and withdrawn animals were conducted within 24 h following their final opioid dose.

2.8. Morphine dose-response curve

Morphine analgesic potency was determined using cumulative dose-response curves, with male mice all receiving successive escalating doses of morphine (0.1, 3, 10, 30 mg/kg, i.p.). Tail withdrawal latencies were measured once every 20 min following each successive dose. EC50 values for all animals were estimated by fitting a non-linear dose-response curve based on their responses to successive doses.

2.9. Locomotion and somatic behavior in spontaneous withdrawal

For assessment of locomotion and somatic withdrawal behavior, mice were placed in the arena used in the locomotor sensitization protocol and recorded for 20 min. No acclimation to the arena was performed prior to testing to prevent habituation and promote exploratory behavior. Video tracking software (Noldus Ethovision, Leesburg, VA) was used to track locomotor behavior for the duration of the test. Withdrawal-associated events (total jumps and grooming sessions) were scored manually using video recordings taken from the test by a blinded experimenter. Jumping incidence was scored over the entire 20-min recording, and grooming sessions were scored during the last 5 min the animal spent within the arena. Grooming sessions were defined as the animal grooming itself in a front-to-back motion in a single location for >2 s.

2.10. Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 9.3.1. All data was tested for normality, and parametric or non-parametric statistics were used accordingly. Changes in drug-paired chamber time in the CPP paradigm between pre- and post-conditioning were compared within each group using one-sample *t* tests, and paired *t* tests with Sidak's correction for multiple tests were performed within each treatment group for comparing saline- and drug-paired chambers. For all other experiments, group means were analyzed using 2- or 3-way analysis of variance (ANOVA) followed by Sidak/Sidak-Holm post-hoc analysis to correct for multiple comparisons, or Dunnett's post-hoc test for comparisons of multiple groups to a single control. All experiments used exclusively male animals apart from the experiments represented in Fig. 4A, C, and D, which used an equal number of male and female mice.

As no effect of sex was observed in preliminary analyses of data in Fig. 4A and D, male and female animals were pooled together for analysis. The locomotor data in Fig. 4C demonstrated an effect of sex, and as a result data was analyzed as a 3-way ANOVA for the effects of morphine, brexpiprazole, and sex. As sex did not significantly influence either treatment, sexes were pooled for post-hoc analysis and figure representations. All data are represented as mean ± S.E.M. Significance threshold was set at $p < 0.05$ for all experiments.

3. Results

3.1. Brexpiprazole attenuates locomotor sensitization in an open-field test

Chronic opioid use is associated with the development of behavioral sensitization to the effects of the drug. (Robinson and Berridge, 2001). Increases in the hyperlocomotor effects of opioids with repeated administration is commonly used to measure the degree to which behavioral sensitization has occurred. We measured the increase in locomotion in mice treated with 40 mg/kg morphine every 72 h in the presence or absence of brexpiprazole over the course of five sessions in an open field apparatus. As expected, locomotor activity increased following repeated injections to morphine (Fig. 1, morphine × session interaction: $F(4, 109) = 68.5$, $p < 0.0001$)). While concomitant treatment of brexpiprazole did not significantly alter the initial locomotor effects of morphine during session 1, brexpiprazole-treated mice displayed attenuated locomotor sensitization with repeated sessions compared to vehicle-treated mice under the same morphine dosing regimen (session x brexpiprazole × morphine interaction: $F(4, 109) = 3.72$, $p = 0.007$)). Control mice treated with saline in lieu of morphine did not show significantly altered locomotion from session 1 in either the brexpiprazole and vehicle groups.

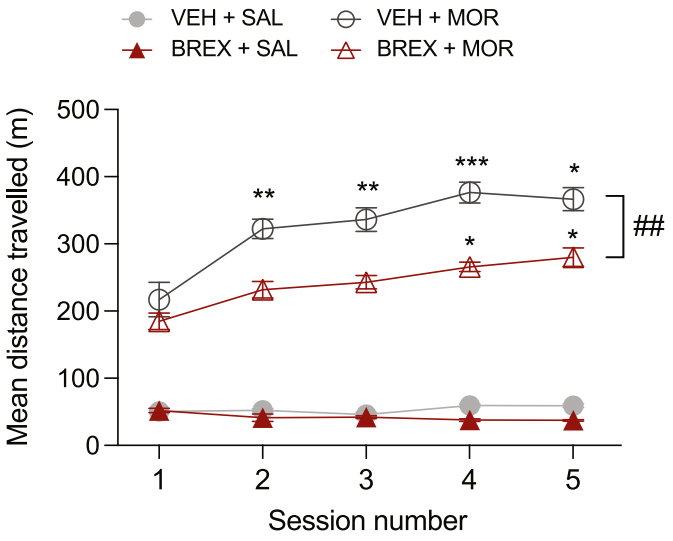

Fig. 1. Brexpiprazole attenuates locomotor sensitization to morphine. Mice were treated with brexpiprazole (0.1 mg/kg, s.c.) or vehicle (3.4% DMSO) 30 min prior to either a saline or morphine (40 mg/kg, i.p.) injection. Mice were put into an open field arena directly following the saline/morphine dose, and distance travelled within the arena was recorded for 30 min. Data are represented as mean with S.E.M., $n = 7$- 8 per group. Groups were compared using three-way ANOVA followed by Sidak-Holm post-hoc comparisons. *p < 0.05, **p < 0.01, ***p < 0.001, session 1 vs. subsequent sessions; ##p < 0.01, interaction between session, morphine, and brexpiprazole.

3.2. Brexpiprazole prevents state-independent, but not state-dependent, expression of morphine CPP

Modulations in dopaminergic transmission, especially D2 receptor (D2R) activation, are involved in pathological drug-seeking in addiction and drug craving during withdrawal (Nestler, 2005). In CPP, animals are trained to associate distinct chambers with either a neutral or rewarding (conditioned) stimulus, and the amount of time spent by the animal in either chamber when given free access to both is indicative of the incentive properties of the paired stimulus. Therefore, time spent in the reward (drug) paired chamber is often used to measure the degree to which the animal experiences the paired drug as rewarding and exhibits drug-seeking behavior. In our CPP paradigm, mice were conditioned to morphine- and saline-paired chambers, before undergoing a state-independent (no morphine given prior to entering chambers) and state-dependent (morphine given prior to chamber entry) test with concomitant pretreatment of either brexpiprazole or vehicle (Fig. 2A). Brexpiprazole prevented the expression of morphine CPP in a state-independent test following conditioning training (Fig. 2B, right; vehicle: $t(15) = 2.2$, $p = 0.01$; brexpiprazole: $t(14) = 0.9$, $p = 0.20$) but failed to block morphine CPP during a state-dependent test in the presence of the drug (Fig. 2C, right; vehicle: $t(15) = 2.8$, $p = 0.01$; brexpiprazole: $t(14) = 3.0$, $p = 0.01$).

3.3. Brexpiprazole blocks reinstatement of morphine CPP following extinction

Following morphine conditioning and post-conditioning tests, all mice underwent 4 days of extinction training to extinguish prior drug-chamber preference. By day 4, no preference for the drug-paired chamber was observed in either group (time in the drug-paired chamber in each group: $t(29) = 0.03$, $p = 0.74$). Reinstatement of CPP following extinction by re-administration of the conditioning drug has previously been used to model drug relapse following abstinence (Mueller et al., 2002; Lu et al., 2003). To determine the effects of brexpiprazole on the reinstatement of morphine CPP, all mice were treated with either vehicle or brexpiprazole prior to a priming dose of 10 mg/kg morphine to reinstate drug preference. Following this priming dose of morphine, mice were placed in the CPP apparatus, and the time spent in each chamber was recorded. Brexpiprazole-treated mice failed to show the significant reinstatement of morphine-paired chamber preference that was observed in vehicle-treated mice (Fig. 2D, right; vehicle: $t(15) = 2.6$, $p = 0.03$; brexpiprazole: $t(14) = 0.6$, $p = 0.56$)).

3.4. Brexpiprazole does not impact the development of morphine analgesic tolerance

Chronic opioid exposure is associated with the development of tolerance to the antinociceptive effects of morphine (Cahill et al., 2016). Given that brexpiprazole was able to attenuate morphine-induced behavioral sensitization and CPP, we next tested to see if brexpiprazole treatment would impact the development of tolerance to the antinociceptive effects of chronic morphine. To induce analgesic tolerance, mice were treated with escalating doses of morphine (10, 20, 30 mg/kg, i.p.) or saline twice daily over three days, before being given a test dose of 10 mg/kg morphine on day 4 (Fig. 3A). All morphine-treated mice in both the vehicle and brexpiprazole groups demonstrated analgesic tolerance during the test dose compared to the 10 mg/kg day 1 dose (morphine × day interaction: $F(4, 112) = 178.4$, $p < 0.0001$). Acute brexpiprazole treatment did not alter the latency to tail withdrawal in response to 10 mg/kg morphine in the morphine groups on day 1 compared to vehicle-treated mice (Fig. 3A, day 1 vehicle-morphine vs. brexpiprazole-morphine). Additionally, mice in the brexpiprazole-saline group had similar responses to the 10 mg/kg test dose on day 4 following three days of brexpiprazole treatment compared to vehicle-saline mice, demonstrating that both acute and chronic brexpiprazole did not impact acute morphine antinociception. Brexpiprazole treatment did not block the development of analgesic tolerance over the course of 4 days compared to vehicle-treated mice (day x morphine × brexpiprazole interaction: $F(4, 112) = 0.8$, $p = 0.70$), nor did brexpiprazole treatment alone alter tail-withdrawal latency (day × brexpiprazole interaction: $F(4, 112) = 1.7$, $p = 0.15$). Analgesic potency was further assessed with a morphine dose-response curve following chronic morphine (10–40 mg/kg over 4 days) or saline injection with concomitant daily brexpiprazole or vehicle (Fig. 3B). As expected, morphine-treated groups demonstrated decreased potency of morphine compared to saline-treated groups, represented by an increase in EC50 (Fig. 3C; effect of morphine: $F(1, 28) = 40.6$, $p < 0.0001$; morphine × brexpiprazole interaction: $F(1, 28) = 0.6$, $p = 0.50$). No significant effect of brexpiprazole was seen in either the saline- or morphine-treated groups (effect of brexpiprazole: $F(1, 28) = 3.6$, $p = 0.07$).

3.5. Acute brexpiprazole treatment attenuates the expression of OIH, but not somatic symptoms of withdrawal

In addition to the emergence of antinociceptive tolerance, chronic opioid exposure generates a paradoxical opioid-induced hyperalgesia during drug-abstinent periods that can be observed as a decrease in sensory thresholds that is revealed following cessation of opioid use (Chu et al., 2008). Mice were made opioid-dependent or received saline in lieu of drug, alongside concomitant brexpiprazole or vehicle, over the course of 4 days. On the fifth day, mice received either vehicle or brexpiprazole in the absence of morphine prior to a thermal tail-withdrawal assay (Fig. 4A). Vehicle-treated mice who received chronic morphine injections displayed a decrease in latency to tail-withdrawal relative to their vehicle and saline-treated counterparts, indicative of the development of hyperalgesia (effect of morphine: $F(1, 28) = 10.07$, $p = 0.003$; effect of brexpiprazole: $F(1, 28) = 0.96$, $p = 0.335$; interaction: $F(1,28) = 0.56$, $p = 0.24$). In the brexpiprazole group, morphine-treated mice failed to show a significantly decreased tail withdrawal latency compared to saline-treated mice following post-hoc analysis. When this experiment was repeated without vehicle

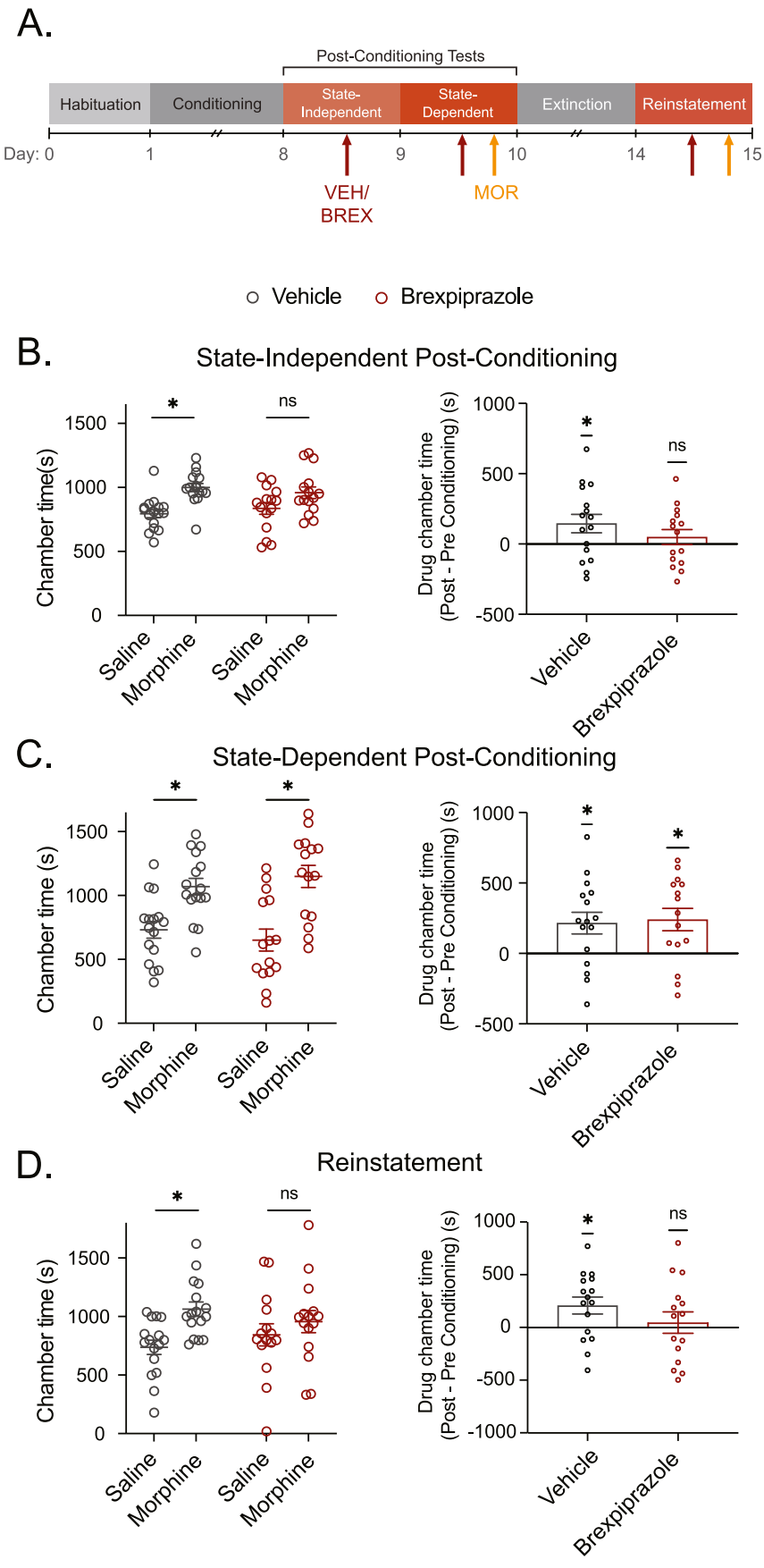

Fig. 2. Brexpiprazole prevents state-independent morphine CPP, and blocks reinstatement following extinction. (A) Timeline of morphine conditioned place preference (CPP) experiment. Mice received 8 days of alternating saline or morphine (10 mg/kg, i.p.) injections in their assigned chambers during the conditioning stage, followed by a state-independent test (day 9) where vehicle or brexpiprazole (0.1 mg/kg, s.c.) (red arrows) was given in the absence of morphine. A state-dependent test (day 10) was performed the following day, where vehicle or brexpiprazole was given in the presence of morphine (10 mg/kg, i.p.) (orange arrows). Following the post-conditioning tests, four days of drug-free extinction training was done to extinguish morphine CPP. A reinstatement test was performed post-extinction by giving all animals either vehicle or brexpiprazole followed by a priming dose of 10 mg/kg morphine. (B, C, D) Time spent in the saline- and drug-paired chambers during the post-conditioning or reinstatement tests (left) and comparisons of the drug chamber time in each test compared to pre-conditioning preferences (right). Grouped data are represented as mean and S.E.M., $n = 16$ per group. Mean time spent in each chamber for each treatment group was compared using t tests followed by Sidak's correction for multiple comparisons, and mean drug chamber time compared to pre-conditioning preferences for each treatment group was analyzed using one-sample t tests; *p < 0.05,.

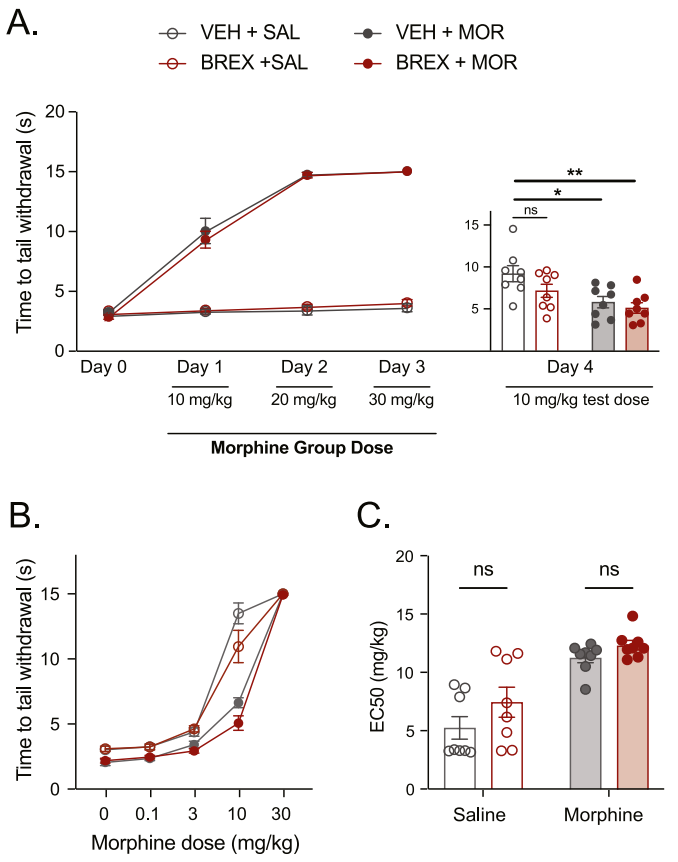

Fig. 3. Brexpiprazole does not impact the development of analgesic tolerance. (A) Tail-withdrawal latencies at baseline (day 0), and across 3 days of treatment (days 1–3), during which animals received either twice-daily saline (i.p.) or escalating morphine (10–30 mg/kg, i.p.) with concomitant treatment of either brexpiprazole (0.1 mg/kg, s.c.) or vehicle. All mice received a test dose of 10 mg/kg on day 4 prior to a final tail-withdrawal assay. Group means across days 0–3 were compared using 3-way ANOVA; day 4 group means were compared using 2-way ANOVA followed by Dunnett's post-hoc test. *$p < 0.05$, **$p < 0.01$ compared to the vehicle-saline group. (B) Morphine dose-response curve performed on mice who had received either 4 days of twice-daily escalating morphine (10–40 mg/kg, i.p.) or saline, with concomitant vehicle or brexpiprazole treatment. (C) Calculated morphine EC50 values for individual animals in the dose-response curve in (B). Group means for EC50 values were compared using two-way ANOVA followed by Sidak's post-hoc comparisons. All data is represented as mean and S.E.M., $n = 8$ per group.

or brexpiprazole pretreatment on the fifth day, morphine-treated mice in both the vehicle- and brexpiprazole-treated groups displayed the same decrease in tail-withdrawal latency relative to their saline controls (Fig. 4B; effect of morphine: $F(1, 28) = 25.5$, $p < 0.0001$; effect of brexpiprazole: $F(1, 28) = 0.05$, $p = 0.82$; interaction: $F(1, 28) = 0.0017$, $p = 0.97$). This indicates that while brexpiprazole does not *prevent* the development of opioid-induced hyperalgesia, acute brexpiprazole is able to attenuate the expression of opioid induced hyperalgesia.

Given that brexpiprazole attenuated the expression of opioid-induced hyperalgesia in the paradigm represented in Fig. 4A, we examined whether brexpiprazole would impact any of the somatic symptoms of acute spontaneous opioid withdrawal. Spontaneous opioid withdrawal in mice is associated with decreased locomotion with an increase in jumping and grooming behavior (Papaleo and Contarino, 2006; McDevitt et al., 2021). When given in the absence of morphine on the first day following an escalating morphine dosing regimen, brexpiprazole failed to prevent the withdrawal-induced decrease in locomotion during an open-field test (Fig. 4C; effect of morphine: $F(1, 24) = 15.7$, $p = 0.0006$; effect of brexpiprazole: $F(1, 24) = 1.47$, $p = 0.24$; morphine × brexpiprazole interaction: $F(1, 24) = 0.047$, $p = 0.83$). Female mice in general displayed more locomotion in the open field arena (effect of sex: $F(1, 24) = 7.72$, $p = 0.011$), consistent with previous reports (Bravo et al., 2020), but sex did not influence the effects of either morphine and/or brexpiprazole treatment (sex × brexpiprazole interaction: $F(1, 24) = 0.013$, $p = 0.91$; sex × morphine interaction: $F(1, 24) = 0.38$, $p = 0.54$; sex x morphine × brexpiprazole interaction: $F(1, 24) = 0.33$, $p = 0.57$). As opioid-withdrawn animals with higher jumping incidence also had lower grooming incidence compared to others within their group, we assessed both withdrawal-associated jumping and grooming events pooled together for all animals (Fig. 4D). Overall, morphine pretreatment significantly increased withdrawal-associated behaviors (effect of morphine: $F(1, 28) = 12.84$, $p = 0.0013$), and while the overall effect of brexpiprazole did not reach significance (effect of brexpiprazole: $F(1, 28) = 3.80$, $p = 0.061$; morphine × brexpiprazole interaction: $F(1, 28) = 2.09$, $p = 0.16$), post-hoc analysis demonstrated a significant increase in withdrawal-associated behaviors in brexpiprazole-morphine mice compared to vehicle-morphine mice ($p = 0.046$).

4. Discussion

Both drug-taking and drug-withdrawal phases in addiction are characterized by altered dopamine neuronal activity and shifts in dopamine homeostasis within the mesolimbic system, resulting in alternating periods of either excessive or limited dopamine availability (Koob and Volkow, 2010; Robinson and Berridge, 2001). As a result, adjunct therapies for drug addiction that can stabilize dopamine transmission by dually lending themselves to both the attenuation or facilitation of dopamine receptor activation during either hyper- or hypodopaminergic states may be a more effective strategy than ones that employ full dopamine agonism or antagonism (Moreira and Dalley, 2015). In this study, we demonstrate that the behavioral effects of brexpiprazole on opioid dependence and withdrawal provide a preclinical basis for brexpiprazole's ability to act as a dopamine system stabilizer in addiction. Furthermore, while its predecessor, aripiprazole, has been shown to modulate opioid salience and reward in previous studies, we investigated the ability of brexpiprazole to not only modulate behaviors related to sensitization and drug-seeking in opioid dependence, but in analgesic tolerance and opioid-induced hyperalgesia as well. This is especially important given that the lowered sensory thresholds seen in opioid withdrawal can contribute to the aversive nature of the withdrawal state (Tsui et al., 2016).

In line with previous studies on aripiprazole (xia Li et al., 2009), a single low dose of brexpiprazole did not affect baseline locomotor activity nor did it alter acute morphine-induced hyperlocomotion (Fig. 1). However, brexpiprazole treatment did block the locomotor sensitization effects observed after repeated morphine treatment. Drug-induced locomotion parallels the release of dopamine within reward pathways (Robinson and Berridge, 2008; di Chiara and Imperato, 1988) and locomotor sensitization reflects heightened striatal dopamine release in chronic drug states. Recently, aripiprazole was shown to specifically reduce firing of hyperexcitable mesolimbic dopamine neurons and prevent subsequent excess dopamine release, without affecting normal neuronal activity (Sonnenschein et al., 2019). That brexpiprazole treatment normalized locomotor activity to previously opioid-naïve levels suggests it is similarly effective at attenuating the increase in dopamine neuronal activity that occurs during behavioral sensitization.

The effects of drugs of abuse are strongly linked to the ability of these drugs to produce drug-seeking behavior and subsequent reinstatement of this behavior following drug discontinuation (de Vries et al., 1998; Vanderschuren et al., 1999). Therefore, we investigated the ability of brexpiprazole to modulate morphine-induced CPP, both directly following morphine conditioning and during reinstatement of CPP following extinction (Fig. 2). In our CPP paradigm, brexpiprazole attenuated preference for the morphine-paired chamber in a

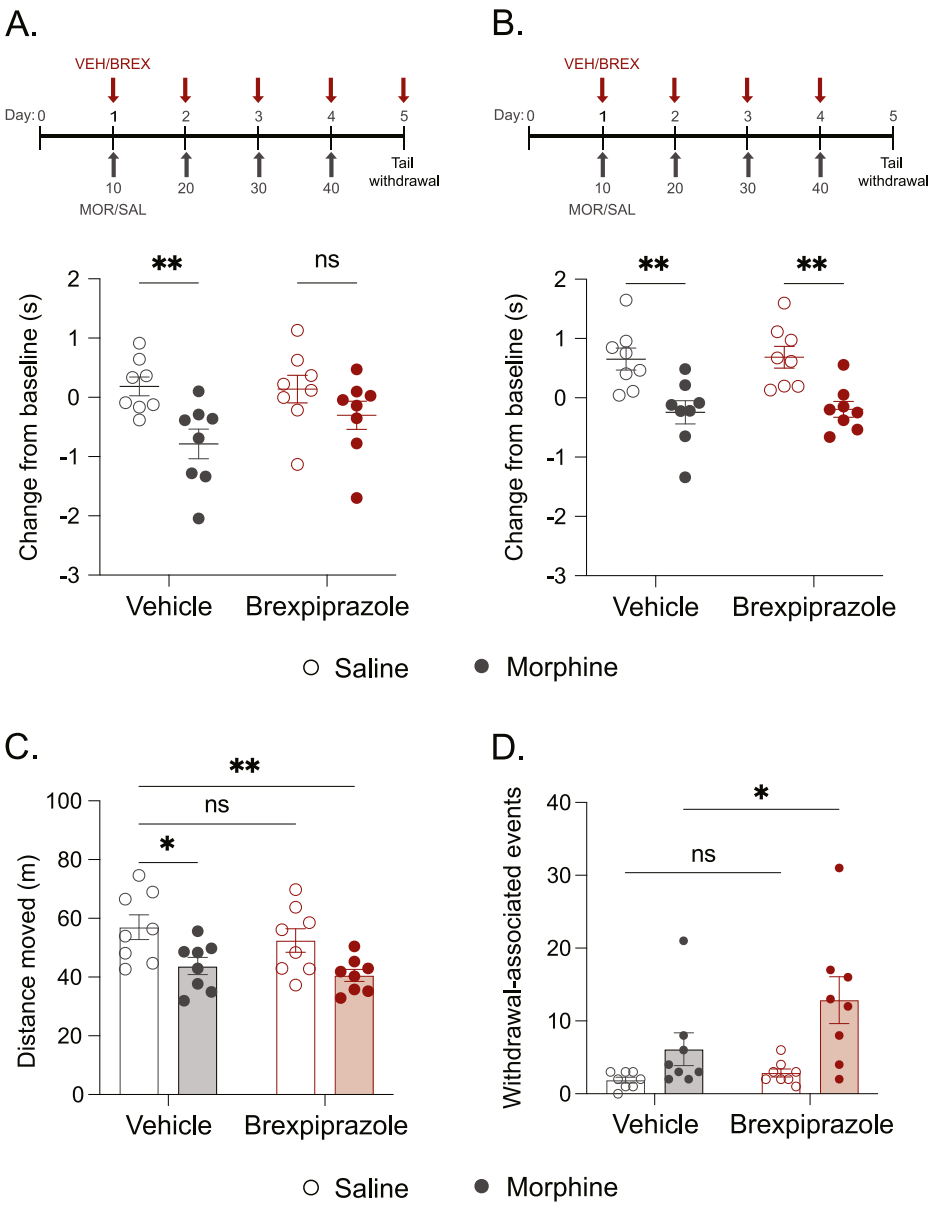

Fig. 4. Acute brexpiprazole treatment attenuates the expression of opioid-induced hyperalgesia. (A) Male and female mice received 4 days of twice daily saline (open circles) or escalating morphine (filled circles), with concomitant daily vehicle (grey) or 0.1 mg/kg brexpiprazole (red). On the fifth day, mice received an injection of either vehicle or 0.1 mg/kg brexpiprazole before thermal sensitivity was measured in a tail withdrawal assay ($n = 8$ per group, $n = 4$ per sex within groups). (B) Male mice received the same 4-day dosing regimen as (A), but did not receive an additional dose of brexpiprazole or vehicle prior to a tail withdrawal assay on the fifth day ($n = 8$ per group). Group means in (A) and (B) were compared using two-way ANOVA followed by Sidak post-hoc tests for multiple comparisons (error bars = S.E.M, *p < 0.05, **p < 0.01). (C, D) Mice from (A) were placed in an arena for a 20-min period during which locomotor activity (C) and withdrawal-associated behaviors (jumping and grooming events) (D) were recorded. Group means in (C) and (D) were compared using 2-way ANOVA followed by Dunnett's (C) or Sidak-Holm (D) post-hoc ($n = 8$ per group, $n = 4$ per sex within groups, error bars = S.E.M, *p < 0.05, **p < 0.01).

state-independent (i.e., in the absence of morphine) post-conditioning test. Interestingly, brexpiprazole failed to alter morphine CPP when this test was performed in a state-dependent manner in the presence of a priming dose of morphine. Other studies have shown that aripiprazole blocks post-conditioning morphine CPP, but only at higher doses (Narita et al., 2008; Almeida-Santos et al., 2014; xia Li et al., 2009), so the failure of brexpiprazole to block morphine CPP when paired with a priming morphine dose may be the result of the insufficiency of the relatively low dose of brexpiprazole used in this study to counteract morphine CPP when strong drug cues are present. Finally, as has previously been observed with aripiprazole (xia Li et al., 2009), brexpiprazole treatment blocked reinstatement of morphine-chamber preference following extinction of the drug-paired chamber preference. Given that the acquisition and expression of morphine CPP is dependent on striatal D2R signalling (Maldonado et al., 1997; Smith et al., 2002; Urs et al., 2011), it is possible that the ability of brexpiprazole to influence drug-seeking behavior in our CPP paradigm reflects its activity as a modulator of dopaminergic signalling via D2R partial agonism.

The failure of brexpiprazole to alter the analgesic properties of morphine, in both an acute antinociceptive test and in the development of analgesic tolerance (Fig. 3), is unsurprising as morphine reward and analgesia are mediated by distinct neural pathways (Gardner, 2011). In line with this, aripiprazole also fails to impact acute morphine antinociception in a tail-withdrawal assay (Almeida-Santos et al., 2014).

Interestingly, concomitant brexpiprazole administration alongside escalating doses of morphine attenuated the expression of opioid-induced hyperalgesia, but only when brexpiprazole was given on the day of testing (Fig. 4). Given that brexpiprazole fails to produce an antinociceptive effect when given alone and did not alter morphine antinociception following either acute or chronic opioid pretreatment (Fig. 3), this is unlikely to be due to a direct antinociceptive effect related to modulation of dopaminergic signalling (Puopolo, 2019). The development of opioid-induced hyperalgesia following opioid discontinuation has been linked to the negative emotional state associated with withdrawal (Edwards et al., 2012; McNally and Akil, 2002), and the hypodopaminergic tone that predominates during withdrawal is likely a major driver of this affective state (Nestler, 2005). As a result, the effects of brexpiprazole on the expression of opioid-induced hyperalgesia may represent an ability to interfere with the affective, as opposed to the nociceptive, component of opioid-induced hyperalgesia. This effect may

be via dopamine receptor stimulation during a hypodopaminergic withdrawal state, or via 5-HT1A partial agonism, as declines in accumbens 5-HT may mediate some of the negative affect experienced during the withdrawal period (Pomrenze et al., 2022). Alternatively, brexpiprazole may aid in the stabilization of spinal dopaminergic and/or serotoninergic transmission, both of which are able to alter nociceptive signalling within the spinal cord (Puopolo, 2019; Wang et al., 2021). This is particularly important to consider given the evidence implicating the spinal cord as a key site in the development and maintenance of opioid-induced hyperalgesia (Roeckel et al., 2016). However, our findings regarding the effects of brexpiprazole on opioid-induced hyperalgesia are limited in part by the fact that we relied on the tail-withdrawal assay, which measures thermal but not mechanical nociception, to assess the expression of pain in withdrawal.

As previously reported, we found that spontaneous opioid withdrawal resulted in a decrease in total locomotor activity in both male and female mice (McDevitt et al., 2021; Bravo et al., 2020). Treatment with brexpiprazole leading up to and during the early withdrawal period did not impact the withdrawal-induced decrease in locomotion. Classically, decreases in locomotor activity in the open-field test are interpreted as anxiety-related behavior, as locomotion in the novel arena is associated with exploratory behavior (Thompson et al., 2015). However, as C57BL/6J mice have been shown to exhibit non-typical anxiety behaviors in the open-field test (Thompson et al., 2015; Mozhui et al., 2010), the extent to which this activity is truly representative of the affective state is questionable. On the other hand, brexpiprazole not only failed to prevent the incidence of jumping and grooming in spontaneous withdrawal, both of which are common somatic symptoms, but rather increased the incidence of these events on the first day post-drug. Given that brexpiprazole has been shown to increase activity of norepinephrine neurons within the locus coeruleus (Oosterhof et al., 2016), it is possible that brexpiprazole may heighten the withdrawal-induced locus coeruleus hyperactivity that underlies some of the somatic symptoms of early acute drug abstinence.

Changes in striatal dopamine release during opioid administration have been shown to be modulated via D2R activity (Rougé-Pont et al., 2002), and the compensatory changes within striatal dopaminergic pathways during drug dependence predominantly involve altered activity in D2-expressing medium spiny neurons (Muntean et al., 2019). The resulting alterations in dopamine signalling play a significant role in driving long-term drug craving and relapse following drug discontinuation (Nestler, 2005). As a partial D2R agonist, brexpiprazole is well-positioned mechanistically to modulate striatal responses to opioid-induced fluctuations in dopaminergic tone, effectively buffering D2R activity in both high- and low-dopamine contexts. Consequently, it largely influences the rewarding, but not analgesic, properties of morphine. However, this does not preclude the possibility that brexpiprazole may exert some of its effects, particularly in opioid-induced hyperalgesia, at the level of the spinal cord. Given that brexpiprazole does not improve, and may indeed exacerbate early somatic symptoms, brexpiprazole treatment is most likely suited to addressing the symptoms of the protracted withdrawal syndrome, such as persistent drug-craving, as opposed to the early acute phase of drug abstinence. In this study, we report that brexpiprazole demonstrates the ability to modulate characteristic dopamine-dependent behaviors in a mouse model of opioid dependence and withdrawal, and therefore may be a useful adjunct therapy in promoting drug abstinence during protracted opioid withdrawal. These results indicate the need for future translational preclinical and clinical investigations into the use of brexpiprazole in opioid use disorder.

CRediT authorship contribution statement

Julia E.R. Nickols: performed the experiments and wrote the manuscript, Writing – original draft, All authors edited and approved the final version of the manuscript. **Serdar M. Dursun:** conceived the project and contributed to experimental design, Project administration. **Anna M.W. Taylor:** conceived the project and contributed to experimental design, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Almeida-Santos, A.F., et al., 2014. The antipsychotic aripiprazole selectively prevents the stimulant and rewarding effects of morphine in mice. Eur. J. Pharmacol. 742, 139–144. https://doi.org/10.1016/J.EJPHAR.2014.09.004. Nov.

Amada, N., et al., 2019. Brexpiprazole has a low risk of dopamine D2 receptor sensitization and inhibits rebound phenomena related to D2 and serotonin 5-HT2A receptors in rats. Neuropsychopharmacol Rep 39 (4), 279. https://doi.org/10.1002/NPR2.12076. Dec.

Berridge, K.C., 2007. The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology (Berl) 191 (3), 391–431. https://doi.org/10.1007/s00213-006-0578-x.

Bravo, I.M., et al., 2020. Divergent behavioral responses in protracted opioid withdrawal in male and female C57BL/6J mice. Eur. J. Neurosci. 51 (3), 742–754. https://doi.org/10.1111/EJN.14580. Feb.

Cahill, C.M., Walwyn, W., Taylor, A.M.W., Pradhan, A.A.A., Evans, C.J., 2016. Allostatic mechanisms of opioid tolerance beyond desensitization and downregulation. Trends Pharmacol. Sci. 37 (11), 963. https://doi.org/10.1016/J.TIPS.2016.08.002. Nov.

Chu, L.F., Angst, M.S., Clark, D., 2008. Opioid-induced hyperalgesia in humans: molecular mechanisms and clinical considerations. Clin. J. Pain 24 (6), 479–496. https://doi.org/10.1097/AJP.0b013e31816b2f43.

Coles, A.S., Knezevic, D., George, T.P., Correll, C.U., Kane, J.M., Castle, D., 2021. Long-acting injectable antipsychotic treatment in schizophrenia and Co-occurring substance use disorders: a systematic review. Front. Psychiatr. 12, 808002 https://doi.org/10.3389/FPSYT.2021.808002. Dec.

Contet, C., Filliol, D., Matifas, A., Kieffer, B.L., 2008. Morphine-induced analgesic tolerance, locomotor sensitization and physical dependence do not require modification of mu opioid receptor, cdk5 and adenylate cyclase activity. Neuropharmacology 54 (3), 475–486. https://doi.org/10.1016/J.NEUROPHARM.2007.10.015. Mar.

Das, S., Barnwal, P., A, B.W., Mondal, S., Saha, I., 2016. Brexpiprazole: so far so good. Ther Adv Psychopharmacol 6 (1), 39. https://doi.org/10.1177/2045125315614739. Feb.

de Vries, T.J., Schoffelmeer, A.N.M., Binnekade, R., Mulder, A.H., Vanderschuren, L.J.M.J., 1998. Drug-induced reinstatement of heroin- and cocaine-seeking behaviour following long-term extinction is associated with expression of behavioural sensitization. Eur. J. Neurosci. 10 (11), 3565–3571. https://doi.org/10.1046/J.1460-9568.1998.00368.X. Nov.

di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA 85 (14), 5274–5278. https://doi.org/10.1073/PNAS.85.14.5274. Jul.

Edwards, S., et al., 2012. Development of mechanical hypersensitivity in rats during heroin and ethanol dependence: alleviation by CRF1 receptor antagonism. Neuropharmacology 62 (2), 1142–1151. https://doi.org/10.1016/J.NEUROPHARM.2011.11.006. Feb.

Frampton, J.E., 2019. Brexpiprazole: a review in schizophrenia. Drugs 79, 189–200. https://doi.org/10.1007/s40265-019-1052-5.

Gardner, E.L., 2011. Introduction: addiction and brain reward and anti-reward pathways. Adv. Psychosom. Med. 30, 22. https://doi.org/10.1159/000324065. Apr.

Kane, J.M., et al., 2016. Overview of short- and long-term tolerability and safety of brexpiprazole in patients with schizophrenia. Schizophr. Res. 174 (1–3), 93–98. https://doi.org/10.1016/J.SCHRES.2016.04.013. Jul.

Kikuchi, T., Maeda, K., Suzuki, M., Hirose, T., Futamura, T., McQuade, R.D., 2021. Discovery research and development history of the dopamine D2 receptor partial agonists, aripiprazole and brexpiprazole. Neuropsychopharmacol Rep 41 (2), 134–143. https://doi.org/10.1002/NPR2.12180. Jun.

Koob, G.F., 2020. Neurobiology of opioid addiction: opponent process, hyperkatifeia, and negative reinforcement. Biol. Psychiatr. 87 (1), 44–53. https://doi.org/10.1016/J.BIOPSYCH.2019.05.023. Jan.

Koob, G.F., Volkow, N.D., 2010. Neurocircuitry of addiction, 35:1 Neuropsychopharmacology 35 (1), 217–238. https://doi.org/10.1038/npp.2009.110. Aug. 2009.

Leite, J.v., Guimarães, F.S., Moreira, F.A., 2008. Aripiprazole, an atypical antipsychotic, prevents the motor hyperactivity induced by psychotomimetics and psychostimulants in mice. Eur. J. Pharmacol. 578 (2–3), 222–227. https://doi.org/10.1016/J.EJPHAR.2007.09.016. Jan.

Lu, L., Shepard, J.D., Hall, F.S., Shaham, Y., 2003. Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. Neurosci. Biobehav. Rev. 27 (5), 457–491. https://doi.org/10.1016/S0149-7634(03)00073-3.

Ma, M., et al., 2016. Adjunctive treatment of brexpiprazole with fluoxetine shows a rapid antidepressant effect in social defeat stress model: role of BDNF-TrkB signaling. Sci. Rep. 6 https://doi.org/10.1038/SREP39209. Dec.

Maeda, K., et al., 2014a. Brexpiprazole I: in vitro and in vivo characterization of a novel serotonin-dopamine activity modulator. J. Pharmacol. Exp. Therapeut. 350 (3), 589–604. https://doi.org/10.1124/JPET.114.213793.

Maeda, K., et al., 2014b. Brexpiprazole II: antipsychotic-like and procognitive effects of a novel serotonin-dopamine activity modulator. J. Pharmacol. Exp. Therapeut. 350 (3), 605–614. https://doi.org/10.1124/JPET.114.213819.

Maldonado, R., Saiardi, A., Valverde, O., Samad, T.A., Roques, B.P., Borrelli, E., 1997. Absence of opiate rewarding effects in mice lacking dopamine D2 receptors, 388: 6642 Nature 388 (6642), 586–589. https://doi.org/10.1038/41567, 1997.

Martinotti, G., et al., 2022. Atypical antipsychotic drugs in dual disorders: current evidence for clinical practice. Curr. Pharmaceut. Des. 28 (27), 2241–2259. https://doi.org/10.2174/1381612828666220623092853. Jun.

McDevitt, D.S., McKendrick, G., Graziane, N.M., 2021. Anterior cingulate cortex is necessary for spontaneous opioid withdrawal and withdrawal-induced hyperalgesia in male mice, 46:11 Neuropsychopharmacology 46 (11), 1990–1999. https://doi.org/10.1038/s41386-021-01118-y. Aug. 2021.

McKeage, K., 2016. Adjunctive brexpiprazole: a review in major depressive disorder. CNS Drugs 30 (2), 91–99. https://doi.org/10.1007/S40263-016-0320-0/FIGURES/1. Feb.

McNally, G.P., Akil, H., 2002. Role of corticotropin-releasing hormone in the amygdala and bed nucleus of the stria terminalis in the behavioral, pain modulatory, and endocrine consequences of opiate withdrawal. Neuroscience 112 (3), 605–617. https://doi.org/10.1016/S0306-4522(02)00105-7. Jul.

Meini, M., et al., 2011. Aripiprazole and ropinirole treatment for cocaine dependence: evidence from a pilot study. Curr. Pharmaceut. Des. 17 (14), 1376–1383. https://doi.org/10.2174/138161211796150783. Jun.

Milienne-Petiot, M., Geyer, M.A., Arnt, J., Young, J.W., 2017. Brexpiprazole reduces hyperactivity, impulsivity, and risk-preference behavior in mice with dopamine transporter knockdown-a model of mania. Psychopharmacology (Berl) 234 (6), 1017–1028. https://doi.org/10.1007/S00213-017-4543-7. Mar.

Moreira, F.A., Dalley, J.W., 2015. Dopamine receptor partial agonists and addiction. Eur. J. Pharmacol. 752, 112–115. https://doi.org/10.1016/J.EJPHAR.2015.02.025. Apr.

Mozhui, K., et al., 2010. Strain differences in stress responsivity are associated with divergent amygdala gene expression and glutamate-mediated neuronal excitability. J. Neurosci. 30 (15), 5357. https://doi.org/10.1523/JNEUROSCI.5017-09.2010. Apr.

Mueller, D., Perdikaris, D., Stewart, J., 2002. Persistence and drug-induced reinstatement of a morphine-induced conditioned place preference. Behav. Brain Res. 136 (2), 389–397. https://doi.org/10.1016/S0166-4328(02)00297-8. Nov.

Muntean, B.S., Dao, M.T., Martemyanov, K.A., 2019. Allostatic changes in the cAMP system drive opioid-induced adaptation in striatal dopamine signaling. Cell Rep. 29 (4), 946. https://doi.org/10.1016/J.CELREP.2019.09.034. Oct.

Narita, M., et al., 2008. Suppression of dopamine-related side effects of morphine by aripiprazole, a dopamine system stabilizer. Eur. J. Pharmacol. 600 (1–3), 105–109. https://doi.org/10.1016/J.EJPHAR.2008.10.030. Dec.

Nestler, E.J., 2005. Is there a common molecular pathway for addiction? Nat. Neurosci. 8 (11), 1445–1449. https://doi.org/10.1038/NN1578. Nov.

Oosterhof, C.A., El Mansari, M., Bundgaard, C., Blier, P., 2016. Brexpiprazole alters monoaminergic systems following repeated administration: an in vivo electrophysiological study. Int. J. Neuropsychopharmacol. 19 (3), 1–12. https://doi.org/10.1093/IJNP/PYV111. Jun.

Papaleo, F., Contarino, A., 2006. Gender- and morphine dose-linked expression of spontaneous somatic opiate withdrawal in mice. Behav. Brain Res. 170 (1), 110–118. https://doi.org/10.1016/J.BBR.2006.02.009. Jun.

Pomrenze, M.B., et al., 2022. Modulation of 5-HT release by dynorphin mediates social deficits during opioid withdrawal. Neuron 110 (24), 4125–4143. https://doi.org/10.1016/j.neuron.2022.09.024 e6.

Puopolo, M., 2019. The hypothalamic-spinal dopaminergic system: a target for pain modulation. Neural Regen Res 14 (6), 925. https://doi.org/10.4103/1673-5374.250567. Jun.

Robinson, T.E., Berridge, K.C., 2001. Incentive-sensitization and addiction. Addiction 96 (1), 103–114. https://doi.org/10.1046/J.1360-0443.2001.9611038.X.

Robinson, T.E., Berridge, K.C., 2008. The incentive sensitization theory of addiction: some current issues. Phil. Trans. Biol. Sci. 363 (1507), 3137. https://doi.org/10.1098/RSTB.2008.0093. Oct.

Roeckel, L.A., Le Coz, G.M., Gavériaux-Ruff, C., Simonin, F., 2016. Opioid-induced hyperalgesia: cellular and molecular mechanisms. Neuroscience 338, 160–182. https://doi.org/10.1016/J.NEUROSCIENCE.2016.06.029. Dec.

Rougé-Pont, F., Usiello, A., Benoit-Marand, M., Gonon, F., Vincenzo Piazza, P., Borrelli, E., 2002. Changes in extracellular dopamine induced by morphine and cocaine: crucial control by D2 receptors. J. Neurosci. 22 (8), 3293. https://doi.org/10.1523/JNEUROSCI.22-08-03293.2002. Apr.

Smith, J.W., Fetsko, L.A., Xu, R., Wang, Y., 2002. Dopamine D2L receptor knockout mice display deficits in positive and negative reinforcing properties of morphine and in avoidance learning. Neuroscience 113 (4), 755–765. https://doi.org/10.1016/S0306-4522(02)00257-9. Sep.

Sonnenschein, S.F., Gill, K.M., Grace, A.A., 2019. State-dependent effects of the D2 partial agonist aripiprazole on dopamine neuron activity in the MAM neurodevelopmental model of schizophrenia. Neuropsychopharmacology 44 (3), 572–580. https://doi.org/10.1038/s41386-018-0219-1.

Strang, J., et al., 2020. Opioid use disorder, 6:1 Nat. Rev. Dis. Prim. 6 (1), 1–28. https://doi.org/10.1038/s41572-019-0137-5. Jan. 2020.

Szerman, N., et al., 2020. Once-monthly long-acting injectable aripiprazole for the treatment of patients with schizophrenia and Co-occurring substance use disorders: a multicentre, observational study. Drugs Real World Outcomes 7 (1), 75–83. https://doi.org/10.1007/S40801-020-00178-8/FIGURES/2. Mar.

Thompson, T., Grabowski-Boase, L., Tarantino, L.M., 2015. Prototypical anxiolytics do not reduce anxiety-like behavior in the open field in C57BL/6J mice. Pharmacol. Biochem. Behav. 133, 7–17. https://doi.org/10.1016/J.PBB.2015.03.011. Jun.

Tsui, J.I., et al., 2016. Chronic pain, craving, and illicit opioid use among patients receiving opioid agonist therapy. Drug Alcohol Depend. 166, 26–31. https://doi.org/10.1016/J.DRUGALCDEP.2016.06.024. Sep.

Urs, N.M., Daigle, T.L., Caron, M.G., 2011. A dopamine D1 receptor-dependent β-arrestin signaling complex potentially regulates morphine-induced psychomotor activation but not reward in mice, 36:3 Neuropsychopharmacology 36 (3), 551–558. https://doi.org/10.1038/npp.2010.186. Oct. 2010.

Vanderschuren, L.J.M.J., Schoffelmeer, A.N.M., Mulder, A.H., de Vries, T.J., 1999. Dopaminergic mechanisms mediating the long-term expression of locomotor sensitization following pre-exposure to morphine or amphetamine, 143:3 Psychopharmacology 143 (3), 244–253. https://doi.org/10.1007/S002130050943, 1999.

Wang, X.Q., Mokhtari, T., Zeng, Y.X., Yue, L.P., Hu, L., 2021. The distinct functions of dopaminergic receptors on pain modulation: a narrative review. Neural Plast. 2021 https://doi.org/10.1155/2021/6682275.

xia Li, S., Zou, Y., jing Liu, L., Wu, P., Lu, L., 2009. Aripiprazole blocks reinstatement but not expression of morphine conditioned place preference in rats. Pharmacol. Biochem. Behav. 92 (2), 370–375. https://doi.org/10.1016/J.PBB.2009.01.014. Apr.