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Negative allosteric modulation of CB1 cannabinoid receptor signalling decreases intravenous morphine self-administration and relapse in mice

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Abstract

The endocannabinoid system interacts with the reward system to modulate responsiveness to natural reinforcers, as well as drugs of abuse. Previous preclinical studies suggested that direct blockade of CB1 cannabinoid receptors (CB1R) could be leveraged as a potential pharmacological approach to treat substance use disorder, but this strategy failed during clinical trials due to severe psychiatric side effects. Alternative strategies have emerged to circumvent the side effects of direct CB1 binding through the development of allosteric modulators. We hypothesized that negative allosteric modulation of CB1R signalling would reduce the reinforcing properties of morphine and decrease behaviours associated with opioid misuse. By employing intravenous self-administration in mice, we studied the effects of GAT358, a functionally-biased CB1R negative allosteric modulator (NAM), on morphine intake, relapse-like behaviour and motivation to work for morphine infusions. GAT358 reduced morphine infusion intake during the maintenance phase of morphine self-administration under a fixed ratio 1 schedule of reinforcement. GAT358 also decreased morphine-seeking behaviour after forced abstinence. Moreover, GAT358 dose dependently decreased the motivation to obtain morphine infusions under a progressive ratio schedule of reinforcement. Strikingly, GAT358 did not affect the motivation to work for food rewards in an identical progressive ratio task, suggesting that the effect of GAT358 in decreasing opioid self-administration was reward specific. Furthermore, GAT58 did not produce motor ataxia in the rotarod test. Our results suggest that CB1R NAMs reduced the reinforcing properties of morphine and could represent a viable therapeutic route to safely decrease misuse of opioids.

KEYWORDS
cannabinoid receptor 1, morphine, motivation, negative allosteric modulator, opioids, reinforcement, self-administration

Chemical compounds: DMSO (PubChem CID: 679), ethanol (PubChem CID: 702), Alkamuls EL-620 (PubChem CID: 482024181), morphine hydrochloride (PubChem CID: 5464110).

1 | INTRODUCTION

Opioid use disorder (OUD) remains at epidemic levels, affecting 16 million people and causing over 120,000 deaths annually from opioid overdose worldwide.[1] Current medications for OUD that target the μ-opioid receptor, such as methadone or buprenorphine, are highly effective and life-saving medications. However, the use of these treatments also faces numerous challenges, including requirements for certified medical personnel, licences, prior authorizations and logistical hurdles.[2] Additionally, these treatments are not risk free or successful in all patients.

μ-Opioid receptors and cannabinoid 1 receptors (CB1R) are G protein-coupled receptors (GPCRs) that are distributed in many of the same areas in the brain, including the classical reward system.[3] Bidirectional interactions between these two GPCRs have been reported on reward, tolerance and other addiction-related behaviours.[4,5] These observations have prompted a growing interest in targeting the endocannabinoid system as a potential avenue for developing alternative and/or adjunct treatments in OUD. In preclinical studies, CB1R antagonists rimonabant (SR141716A) and AM251 reduced consumption of several drugs of abuse in rodents, including psychostimulants, opioids, alcohol and nicotine.[5] However, when rimonabant was used as an anti-obesity drug in people, adverse psychiatric side effects led to its withdrawal from the market and the termination of clinical programmes on CB1 antagonists/inverse agonists. Since then, alternative strategies have emerged to circumvent the negative outcomes of direct CB1R binding through the development of allosteric modulators. Currently, very few preclinical studies have examined the therapeutic effects of CB1R negative allosteric modulators (NAMs) in drug addiction. The two most studied allosteric modulators of CB1R receptors are Org27569 and PSNCBAM-1. Org27569 attenuated cue- and drug-induced reinstatement of cocaine- and methamphetamine-seeking behaviour in rats.[6] PSNCBAM-1 attenuated the reinstatement of extinguished cocaine-seeking behaviour in rats.[7] However, questions have been raised regarding their selectivity given that, like rimonabant, Org27569 and PSNCBAM-1 caused anhedonia, decreasing food intake.[8,9] Furthermore, both Org27569 and PSNCBAM-1 displayed a contradictory pharmacological profile, acting as an inverse agonist while also acting as a NAM of CB1R depending on the orthosteric agonists active in a given circuit.[10–12]

We recently developed a novel CB1R NAM, GAT358, which displays functional selectivity in the β-arrestin assay over the cAMP formation assay (biased NAM) using a focused structure–activity relationship study on PSNCBAM-1.[13] Our previous studies demonstrated that the CB1R NAM GAT358 suppressed morphine reward (i.e., conditioned place preference [CPP]) and attenuated morphine-induced dopamine efflux in the nucleus accumbens (NAc) shell.[14] However, no prior research has examined the effects of a CB1 NAM on opioid reinforcement using translationally relevant operant behaviour paradigms (that is, intravenous self-administration, which involve drug, context, cues and an action that is performed to obtain the drug).

In the present work, we investigated the effects of the CB1R NAM GAT358 on the reinforcing properties of morphine in mice by employing intravenous drug self-administration, the gold standard for studying drug addiction. Our results collectively suggest that the CB1R NAM GAT358 reduces the reinforcing properties of morphine. CB1 NAMs such as GAT358 may represent a viable therapeutic route to decrease opioid addictive behaviours and relapse while circumventing the adverse side effects of CB1R orthosteric agonists.

2 | MATERIALS AND METHODS

2.1 | Drugs

GAT358 was synthesized in the laboratory of Ganesh Thakur in the Department of Pharmaceutical Sciences at Northeastern University (by LNC). GAT358 was dissolved in a vehicle (VEH) containing 20% dimethyl sulfoxide (DMSO), 8% ethanol, 8% Alkamuls EL-620 and 64% saline and administered intraperitoneally at 10, 20 or 30 mg/kg in a final volume of 5 mL/kg injection. Dose selection was based on our previous studies showing that 20 mg/kg (ip) decreased morphine-induced increases in dopamine efflux in the NAc, blocked morphine-induced CPP and decreased the oral consumption of oxycodone.[14] Morphine hydrochloride was obtained from the National Institute on Drug Abuse (NIDA), dissolved in a physiological saline solution and administered at 300 μg/kg/infusion in the intravenous drug self-administration paradigm.

2.2 | Animals

All procedures were approved by the Institutional Animal Care and Use Committee at the Indiana University Bloomington. Adult C57BL/6J male mice (The Jackson Laboratory, Bar Harbor, ME), 15 weeks old at the start of the study, were group housed upon arrival, given ad libitum access to water/food and maintained on a 12-h reversed light/dark cycle. Mice were subjected to at least 48 h of acclimation to the animal facilities prior to starting any experimental procedure. After the acclimation period, all mice were single housed for the duration of the experiments. All experiments were performed during the dark phase of the cycle. A total of 79 animals were used in this study. Out of this total, one mouse was excluded due to catheter leakage and four mice were excluded due to failure to meet the minimum infusion criteria described in Section 2.4; none of the excluded animals ever received any intraperitoneal pharmacological manipulations of GAT358 or VEH.

2.3 | Jugular catheter surgery

Surgeries were performed under isoflurane anaesthesia (2% to 3%, 1.5 L/min O_2). A back mount cannula (315BM-8-5UP, P1 technology, Roanoke, VA) was connected to a 4 cm micro-renathane tubing (0.025-in. outer diameter, 0.012-in. inner diameter, Braintree Scientific Inc.) and a silicon bead (kit-silicone low viscosity, WPI, Sarasota,

FL) was placed approximately 10 mm from the tip.[15] Briefly, tubing was passed subcutaneously from the back of the mouse to the neck, inserted into the jugular vein and surgical silk sutures were used to anchor the catheter to the jugular vein above and below the silicone bead. We infused heparinized saline (20 μL, 100 unit/mL) and withdrew blood from the jugular vein to ensure correct catheter placement and patency. Incisions were closed using tissue adhesive (Vetbond, 3M). Mice were allowed to recover for 1 week following the catheter surgery before starting the morphine self-administration sessions. Catheters were flushed daily with the same heparinized saline solution before and after each drug self-administration session to detect any potential fluid leakage or changes in fluid flow resistance.

2.4 | Intravenous morphine self-administration

Animals were tested once daily in 120 min drug self-administration-sessions. Operant chambers (Med Associates Inc., St. Albans, VT) were equipped with grid floors, a house light, left and right nose poke ports and an infusion pump. A morphine dose of 300 μg/kg/infusion was used for these experiments.[16] Each trial started with the house light ON and the guillotine doors opened, allowing access to the nose poke ports. Meeting the schedule of reinforcement on the active nose poke port resulted in the house light turning off and delivery of one morphine infusion paired with the active nose poke light enabled (i.e., as a discrete cue signalling morphine delivery). Actions in the inactive nose poke port had no scheduled outcomes. For a typical 30 g mouse, the infusion pump and the nose poke light turned on for approximately 2 s and 3.6 μL of morphine solution (2.5 mg/mL) were infused. Infusion volume was adjusted based on each subject's weight to maintain the same drug infusion dose. Each infusion was followed by a 30 s timeout period where actions on either nose pokes port did not produce any consequence. In our self-administration experimental design, mice were never pre-trained to nose poke for food, and consequently, the drug consumption in our experiments is truly volitional. All mice in intravenous morphine self-administration studies were maintained on ad libitum food for the entire duration of the experiment.

In the maintenance experiment, mice were first allowed to self-administer morphine for 10 consecutive days on a FR1 schedule of reinforcement (the acquisition phase). Then, mice continued on FR1 until they exhibited stable performance. The criterion for stable morphine intake was reached when the mice received at least 10 infusions per session and exhibited less than 15% variance in the number of infusions for three consecutive days.[17] After meeting this criterion, mice received counterbalanced injections of GAT358 (20 mg/kg ip) or VEH (ip) 20 min before the self-administration session. Mice were allowed to recover to baseline infusion intake between treatments.

In the relapse test, a different group of mice was first allowed to self-administer morphine for 15 days on a FR1 schedule of reinforcement. Animals that achieved at least 10 infusions per session at the end of the 15 days were then subjected to 21 days of forced abstinence in their home cages followed by a single cue-induced drug-seeking session (i.e., relapse test) in the operant chambers. To minimize experimenter-induced stress on the relapse test day, mice were handled daily during the forced abstinence period. Mice were treated with GAT358 (20 mg/kg ip) or VEH (ip) 20 min prior to the relapse test session. Subsequently, the subjects were placed back into the drug self-administration operant chambers with an identical configuration as before; however, in the relapse test, active nose poking resulted in the delivery only of the discrete cue previously paired with morphine (i.e., nose poke light), but no morphine was infused.

In the progressive ratio (PR) experiment, a different cohort of mice was trained to self-administer morphine for 10 days on FR1, 5 days on FR2 and 5 days on FR3 schedules of reinforcement. Animals were required to get at least 10 infusions before progressing to the next FR. Next, mice were subjected to a PR schedule of reinforcement where they had to increase their effort in the same session to get an infusion (i.e., PR values were 1, 2, 4, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, 1347, 1647 and 2012). All FR and PR sessions in this study lasted 120 min. Mice continued on PR until achieving a stable intake of morphine. As described above, a stable morphine infusion intake was defined by observation of less than 15% variance in the number of infusions across three consecutive days. In the following PR sessions, mice were treated in a counterbalanced order with GAT358 (10 mg/kg ip), GAT358 (20 mg/kg ip), GAT358 (30 mg/kg ip) or VEH (ip) 20 min before testing. Animals were allowed to recover baseline levels of infusion intake in between treated sessions to avoid any carryover effects between doses.

2.5 | Rotarod test

A separate cohort of otherwise naïve mice was first subjected to 30 min of acclimation to the testing room and subsequently placed on a rotarod apparatus which measures latency of ambulating animals to fall off a rotating drum. Animals underwent a total of two training days and one test day on the rotarod apparatus (IITC Life Sciences Inc., Woodland Hills, CA). The rotarod apparatus was set to start rotating at four rotations per minute (RPM) and accelerate to 40 RPM. A 300 s cut-off was employed. The goal for the training days for each mouse was to stay on the drum of the apparatus for at least 30 s in three consecutive trials with a maximum of six trials a day and a 20 min interval between trials. On the test day, to qualify for testing, each mouse was required to stay on the drum for at least 30 s in a single trial. Animals that did not meet this criterion were excluded from the experiment and were never subjected to pharmacological manipulations. Mice that qualified for testing experienced two baseline trials (i.e., pre-injection) and then were randomly treated with GAT358 (10 mg/kg ip), GAT358 (20 mg/kg ip), GAT358 (30 mg/kg ip) or VEH (ip) 20 min before being subjected to another two trials (i.e., post-injection) to assess latency to descend from the rotating drum.

2.6 | Food self-administration

The VEH-treated animals previously subjected to the rotarod test were subsequently food restricted (1.7–2 g regular chow per day) until they lost 10% of body weight (approximately 1 week). These animals remained in food restriction for the entire duration of the experiment. The operant chambers, trial structure and session duration were identical to those used for drug self-administration. These chambers were also equipped with two nose poke ports, one designated as an active and the other one as an inactive nose poke. Each trial started with the house light ON. Actions in the inactive nose poke produced no consequences whereas meeting the schedule of reinforcement on the active nose poke turned OFF the house light and delivered a 14 g sucrose pellet (BioServ F05684) paired with a nose poke light cue. The reward delivery was followed by a 30 s timeout, during which the house light remained off and actions on either nose pokes had no scheduled outcomes. Once the timeout period concluded, the house light turned on again and a new trial started. Mice were first trained on FR1, FR2 and FR3 schedules of reinforcement with the requirement to earn 60 or more pellets before passing to the next FR. Mice were then trained on PR (i.e., using the same PR values as for drug self-administration) schedule until a stable number of pellets were earned. As before, stable pellet intake was defined as observation of less than 15% variance in the number of pellets earned across three consecutive days. In the following PR sessions, mice received, in a counterbalanced manner, GAT358 (10 mg/kg ip), GAT358 (20 mg/kg ip), GAT358 (30 mg/kg ip) or VEH (ip) 20 min before testing. Animals were allowed to recover previous amounts of food pellet intake in between treated sessions to avoid any carryover effects between doses.

2.7 | Statistical analysis

Data were analysed by one-way or two-way analysis of variance (ANOVA) followed by Dunnett's or Bonferroni's post hoc tests. The Geisser–Greenhouse correction was applied to all repeated factors to adjust the lack of sphericity in repeated measures ANOVA. When appropriate, significant differences between means determined within subjects were assessed using a two-tailed paired *t* test. In a few instances, during the initial FR1, FR2 and FR3 training, a subset of mice chewed on the external tubing in the operant chamber that tethers the mouse to the infusion pump which delivers morphine into the implanted catheter. This led to leakage of morphine from the tubing, which prevented it from reaching the jugular vein, and resulted in an abnormal number of nose pokes. These sessions were excluded from the analysis. In the present study, biting of the external tubing occurred on four occasions involving three different mice in the cohort that underwent FR1, FR2 and FR3 on Sessions 7, 10 and 11, respectively. Note that chewing on the tubing that was tethered to the infusion pump did not affect the viability of the implanted jugular catheter. Thus, no subjects were excluded on the basis of the appearance of this behaviour in a small subset of training trials that never involved intraperitoneal pharmacological manipulations. All statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, CA). Data are presented as mean ± SEM and $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | GAT358 reduces morphine intake during the maintenance phase of self-administration

We first evaluated the impact of GAT358 (20 mg/kg ip) on morphine intake during the maintenance phase of drug self-administration (Figure 1A). During the acquisition phase, morphine infusion intake escalated (Figure 1B) and notably distinguished the reinforced and non-reinforced nose pokes across sessions (Figure 1C). In the maintenance phase, GAT358 (20 mg/kg ip) decreased the number of infusions and the number of active nose pokes whereas VEH (ip) failed to do so (Figure 1D,E). Specifically, GAT358 (20 mg/kg ip) reduced morphine infusion intake (paired *t* test, two-tailed, $p = 0.0399$; infusions [mean ± SEM]: VEH: 43.86 ± 5.13, GAT358: 32.71 ± 6.82, $n = 7$ [counterbalanced]; Figure 1F). Likewise, GAT358 (20 mg/kg ip) decreased the total number of active nose pokes relative to VEH treatment (paired *t* test, two-tailed, $p = 0.0098$, active nose pokes [mean ± SEM]: VEH: 60.43 ± 8.10, GAT358: 39.57 ± 7.15, $n = 7$ [counterbalanced]; Figure 1G). GAT358 (20 mg/kg ip) did not alter the total number of inactive nose pokes relative to VEH (ip) treatment (paired *t* test, two-tailed, $p = 0.312$, inactive nose pokes [mean ± SEM]: VEH: 4.42 ± 1.44, GAT358: 2.42 ± 0.84, $n = 7$ [counterbalanced]; Figure 1H). GAT358 (20 mg/kg ip) decreased the total quantity of morphine consumed compared with VEH treatment (paired *t* test, two-tailed, $p = 0.0288$, $n = 7$ [counterbalanced]; Figure 1I). Similarly, GAT 358 (20 mg/kg ip) reduced the number of cumulative infusions compared with VEH, and these effects were also time dependent (Figure 1J); two-way ANOVA revealed a main effect of time ($F_{24,144} = 25.7$, $p < 0.0001$), and treatment ($F_{1,6} = 6.641$, $p = 0.0419$) and the interaction was significant ($F_{24,144} = 1.665$, $p = 0.0357$). Bonferroni's post hoc multiple comparisons revealed that GAT358 reliably reduced the cumulative number of infusions at 20 ($p = 0.0162$), 25 ($p = 0.0015$) and from 30 to 120 min ($p < 0.0001$) (Figure 1J).

3.2 | GAT358 decreases the relapse of morphine seeking after forced abstinence

Next, we studied the impact of GAT358 (20 mg/kg ip) on the relapse of morphine seeking after 21 days of forced abstinence (Figure 2A). After 15 sessions of drug self-administration, mice successfully acquired morphine intake behaviour (Figure 2B) and accurately discerned the reinforced from the non-reinforced nose poke throughout the 15 self-administration sessions (Figure 2C). After 21 days of forced abstinence, GAT358 (20 mg/kg ip) pre-treatment successfully decreased morphine-seeking behaviours in the relapse test

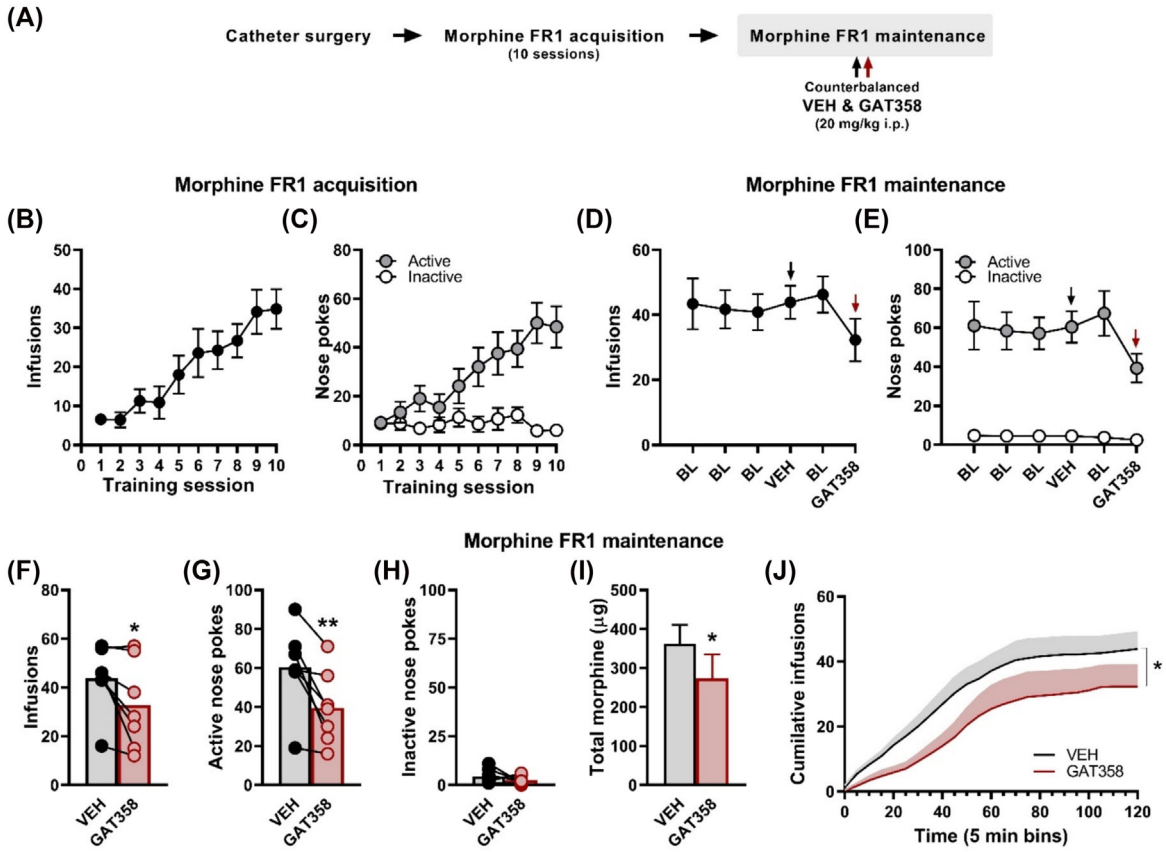

FIGURE 1 GAT358 reduces morphine consumption after the establishment of stable drug infusion intake. (A) Schematic shows the order of the experimental procedures. Mice were implanted with intravenous jugular catheters. After recovering from the surgery, mice were allowed to self-administer morphine on a FR1 schedule of reinforcement for 10 sessions (acquisition phase). Mice were allowed to continue self-administering morphine under FR1 until reaching a stable infusion intake (maintenance phase) (>10 infusions and less than or equal to 15% variance for three consecutive days). Subsequently, all animals received vehicle (VEH, ip, black arrow) and GAT358 (20 mg/kg ip, red arrow) in a counterbalanced manner 20 min before operant sessions. The number of morphine infusions (B) and number of active and inactive nose pokes (C) increased across the initial 10 morphine self-administration sessions comprising the acquisition phase. During the maintenance phase of morphine self-administration, prior to (ip) pharmacological manipulations, the number of morphine infusions increased across sessions (D) and an escalation in the number of active but not inactive nose pokes (E) was observed. After reaching stable baseline (BL) responding for morphine, mice received counterbalanced injections of VEH (ip) and GAT358 (20 mg/kg ip). GAT358 (20 mg/kg ip) decreased the average number of morphine infusions (F), and the average number of active nose pokes (G) but did not alter the average number of inactive nose pokes (H) compared with VEH (ip) treatment during the maintenance phase of self-administration. Note that in the figure, data are normalized to show VEH first, but order of treatments was counterbalanced between animals. (I) GAT358 (20 mg/kg ip) decreased total morphine consumed in the maintenance phase of self-administration relative to VEH (ip). (J) GAT358 (20 mg/kg ip) decreased the cumulative number of infusions of mice in the maintenance phase of morphine self-administration relative to mice that received VEH (ip). Data are expressed as mean ± SEM ($n = 7$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

(Figure 2D). A two-way ANOVA revealed main effects of treatment ($F_{1,40} = 13.53$, $p = 0.0007$) and nose poke type ($F_{1,40} = 34.84$, $p < 0.0001$), and a significant interaction between treatment and the nose poke type ($F_{1,40} = 6.369$, $p = 0.0157$) was also observed. Bonferroni's post hoc multiple comparisons revealed that GAT358 (20 mg/kg ip) decreased the number of active nose pokes ($p = 0.0002$; active nose pokes [mean ± SEM]: VEH [$n = 12$]: 118.41 ± 17.94, GAT358 [$n = 10$]: 45 ± 13.71) compared with the VEH-treated group. By contrast, the number of inactive nose pokes did not differ between groups ($p = 0.8383$; inactive nose pokes [mean ± SEM]: VEH [$n = 12$]: 18.66 ± 3.38 and GAT358 [$n = 10$] 5 ± 1.54) (Figure 2D).

3.3 | GAT358 reduced the motivation to obtain morphine infusions

We evaluated the effects of different doses of GAT358 on the motivation to work for morphine rewards. We tested multiple doses of GAT358 (10, 20 and 30 mg/kg) to permit assessment of dose-dependent differences in the motivation strength of the subjects (Figure 3A). Mice increased the number of infusions taken (Figure 3B) and readily distinguished between active and active nose pokes (Figure 3C) at the end of the FR training. One-way ANOVA, comparing the last two sessions in each FR, showed no differences in the number of infusions between the FR schedules ($F_{2,18} = 0.3042$,

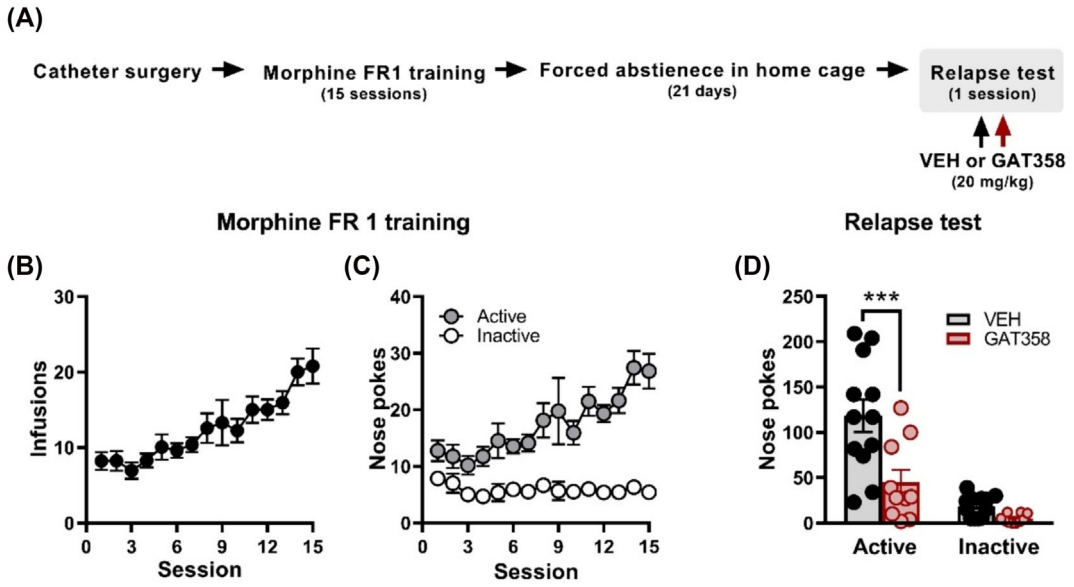

FIGURE 2 GAT358 decreases the relapse of morphine-seeking behaviour after forced abstinence. (A) Schematic shows the order of experimental procedures. Mice were implanted with intravenous jugular catheters. After recovering from the surgery, animals were allowed to self-administer morphine on a FR1 schedule of reinforcement for 15 session and then spent 21 days in forced abstinence in their home cages. Then, mice were tested in a single relapse session in which they received vehicle (VEH, ip, black arrow) or GAT358 (20 mg/kg ip, red arrow) 20 min before behavioural testing. Prior to pharmacological (intraperitoneal) manipulations morphine infusion intake (B) and the number of active, but not inactive, nose pokes (C) increased across sessions during FR1 training. (D) GAT358 (20 mg/kg ip) reduced the number of active but not inactive nose pokes during the relapse test relative to VEH treatment. Data are expressed as mean ± SEM, VEH $n = 12$, GAT358 $n = 10$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

$p = 0.7414$; infusions [mean ± SEM]: FR1: 26.79 ± 4.42, FR2: 23.57 ± 3.25, FR3: 22.64 ± 4.06; $n = 7$ for each FR ratio; Figure 3D). However, the number of active nose pokes was higher ($F_{2,18} = 4.45$, $p = 0.0269$; active nose pokes [mean ± SEM]: FR1: 35.29 ± 4.44, FR2: 50.54 ± 8.19, FR3: 74.14 ± 12.95; $n = 7$ [counterbalanced] for each FR ratio) under FR3 compared with FR1 schedules ($p = 0.0239$; Figure 3E). The number of inactive nose pokes ($F_{2,18} = 4.483$, $p = 0.0263$; inactive nose pokes [mean ± SEM]: FR1: 7.5 ± 1.38, FR2: 5.28 ± 1.15, FR3: 2.78 ± 0.67; $n = 7$ [counterbalanced] for each FR ratio) was similarly lower under the FR3 compared with the FR1 schedule ($p = 0.0234$; Figure 3F). In the PR schedule, GAT358 (10, 20 and 30 mg/kg ip) dose dependently decreased the number of morphine infusions taken (Figure 3G). GAT358 treatment decreased the number of morphine infusions ($F_{1.89,11.38} = 17.68$, $p = 0.0004$; infusions [mean ± SEM]: VEH: 8.28 ± 0.99, GAT358 [10 mg/kg]: 7.14 ± 1.05, GAT358 [20 mg/kg]: 6.28 ± 0.89, GAT358 [30 mg/kg]: 3.85 ± 0.98, $n = 7$ [counterbalanced]; Figure 3G); GAT358 30 mg/kg ip reduced morphine infusion intake compared with either VEH ($p = 0.0004$), GAT358 10 mg/kg ip ($p = 0.0088$) or GAT358 20 mg/kg ip ($p = 0.0145$). Furthermore, we observed a significant negative linear trend between treatments ($p < 0.0001$; Figure 3G). Similarly, GAT358 (10, 20 and 30 mg/kg ip) dose dependently decreased the number of active nose pokes ($F_{1.75,10.51} = 6.487$, $p = 0.0168$; active nose pokes [mean ± SEM]: VEH: 126.9 ± 32, GAT358 [10 mg/kg]: 104.4 ± 34.83, GAT358 [20 mg/kg]: 73.71 ± 19.81 and GAT358 [30 mg/kg]: 35 ± 13.71, $n = 7$ [counterbalanced]; Figure 3H); The high dose of GAT358 (30 mg/kg ip) decreased the number of active nose pokes compared with VEH ($p = 0.0238$) and both the low (10 mg/kg ip; $p = 0.0088$) and middle (20 mg/kg ip) doses of GAT358 ($p = 0.0464$). A negative linear trend was also observed between treatments ($p < 0.0004$; Figure 3H). By contrast, the number of inactive nose pokes remained almost unaltered across the different doses of GAT358 ($F_{1.52,9.16} = 4.37$, $p = 0.0540$; inactive nose pokes [mean ± SEM]: VEH: 10.71 ± 3.92, GAT358 [10 mg/kg]: 11 ± 4.38, GAT358 [20 mg/kg]: 5.57 ± 2.91 and GAT358 [30 mg/kg]: 2.57 ± 1.15, $n = 7$ [counterbalanced]; Figure 3I). GAT358 also decreased the final ratio completed in a dose-dependent manner ($F_{1.67,10.06} = 11.04$, $p = 0.0038$; break point [mean ± SEM]: VEH: 30.86 ± 6.26, GAT358 [10 mg/kg]: 23.71 ± 6.12, GAT358 [20 mg/kg]: 18.43 ± 4.11 and GAT358 [30 mg/kg]: 8.87 ± 3.2, $n = 7$ [counterbalanced]; Figure 3J). The last completed ratio was lower in GAT358 30 mg/kg ip treated groups compared with VEH ($p = 0.0054$), GAT358 10 mg/kg ip ($p = 0.0395$) and GAT358 20 mg/kg ip ($p = 0.0233$) treated groups. A negative linear trend in last completed ratio was also observed between treatments ($p < 0.0001$; Figure 3J). See Table S1 for individual subjects data values.

4 | GAT358 DID NOT PRODUCE MOTOR ATAXIA

GAT358 (10, 20 and 30 mg/kg ip) did not impair motor coordination in the rotarod test (Figure 4A). No differences were detected in

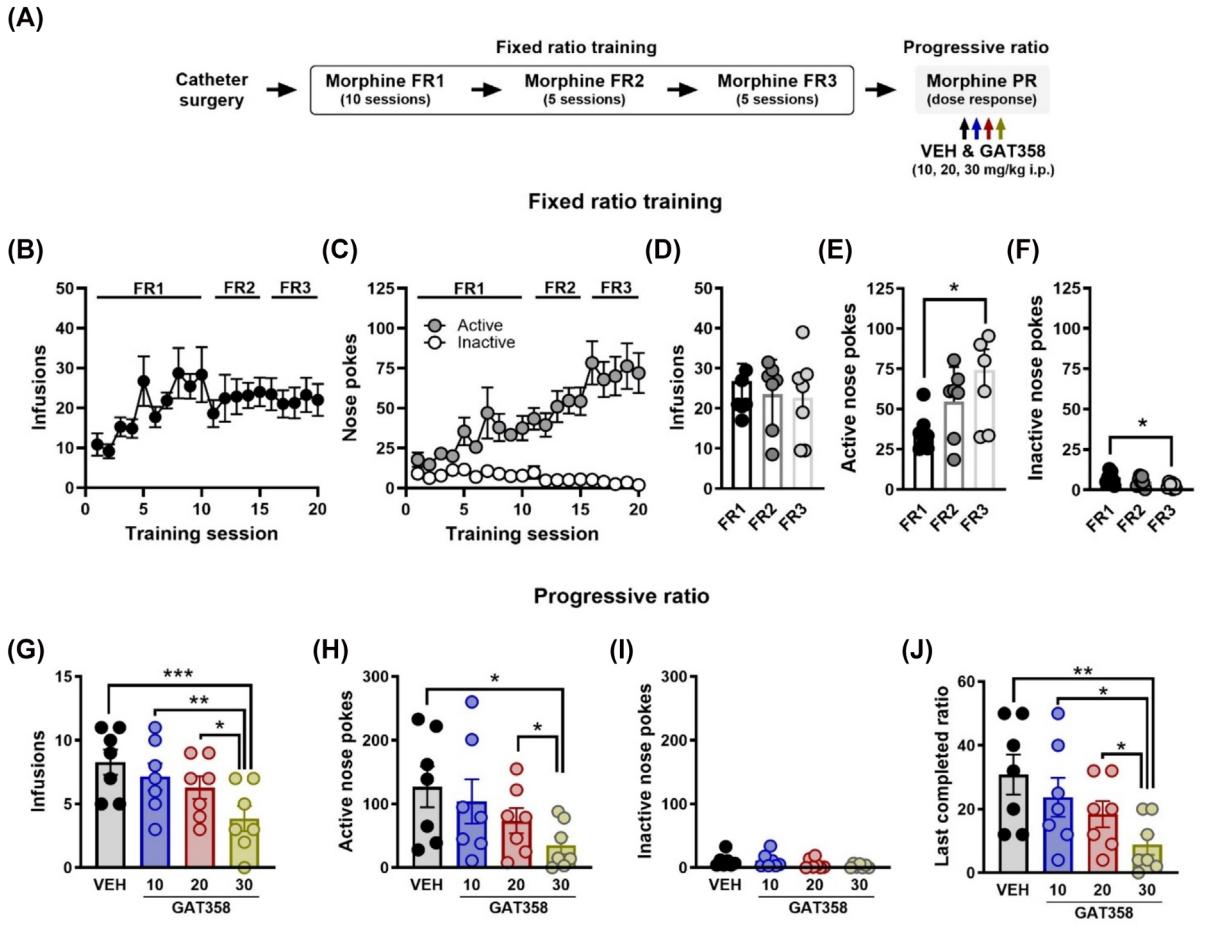

FIGURE 3 GAT358 reduces the motivation to work for morphine rewards in a progressive ratio schedule of reinforcement. (A) Schematic shows the experimental design. Briefly, mice were first trained under FR1 (10 days), FR2 (days) and FR3 (5 days) schedules of reinforcement. Then, mice underwent PR training until reaching stable morphine infusion intake (i.e., less than 15% variance for three consecutive days). After mice acquired stable behaviour under PR, they received counterbalanced injections of vehicle (VEH, ip, black arrow) and GAT358 (10, 20 and 30 mg/kg ip, blue, red and green arrows) before behavioural testing. (B) Infusion intake increased during the fixed ratio training under FR1 and was stable under FR2 and FR3. (C) Active nose pokes were greater than inactive nose pokes during the fixed ratio training. Average number of infusions (D), active nose pokes (E) and inactive nose pokes in the last 2 days of each fixed ratio training (F). GAT358 dose dependently decreased the number of morphine infusions earned (G), the number of active nose pokes (H), but not the number of inactive nose pokes (I) and decreased the last completed ratio for morphine infusion intake (J) during PR schedule of reinforcement. Data are expressed as mean ± SEM ($n = 7$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

latency to descend from the rotating drum in groups treated with either VEH or any dose of GAT358 (10, 20 and 30 mg/kg ip) (Figure 4A,B). Two-way ANOVA did not detect an interaction in descent between pre- and post-injection trials across treatments ($F_{3,27} = 0.9577$, $p = 0.4269$; $n = 7$–8 per group; Figure 4B).

5 | GAT358 DID NOT REDUCE THE MOTIVATION TO WORK FOR FOOD REWARDS

We asked whether GAT358 would alter the self-administration of food rewards (Figure 5A). None of the doses of GAT358 altered the number of pellets consumed ($F_{1.9,11.47} = 0.7913$, $p = 0.4712$; food pellets [mean ± SEM]: VEH: 17.71 ± 0.86, GAT358 [10 mg/kg]: 18.57 ± 0.68, GAT358 [20 mg/kg]: 18.71 ± 0.52 and GAT358 [30 mg/kg]: 18.29 ± 0.74, $n = 7$ [counterbalanced]; Figure 5B), the number of active nose pokes ($F_{1.9,11.65} = 0.3819$, $p = 0.6850$; active nose pokes [mean ± SEM]: VEH: 1363 ± 272, GAT358 [10 mg/kg]: 1585 ± 196.5, GAT358 [20 mg/kg]: 1482 ± 148.4 and GAT358 [30 mg/kg]: 1434 ± 211, $n = 7$ [counterbalanced]; Figure 5C), the number of inactive nose pokes ($F_{1.1,6.8} = 0.4931$, $p = 0.594$; inactive nose pokes [mean ± SEM]: VEH: 29.14 ± 6.92, GAT358 [10 mg/kg]: 22.86 ± 5.3, GAT358 [20 mg/kg]: 39.86 ± 19.91 and GAT358 [30 mg/kg]: 26.43 ± 8.59, $n = 7$ [counterbalanced]; Figure 5D) or the motivation to obtain food rewards ($F_{1.8,11.2} = 0.4463$, $p = 0.6349$; last completed ratio [mean ± SEM]: VEH: 226.3 ± 40.89, GAT358 [10 mg/kg]: 58.1 ± 29.89, GAT358 [20 mg/kg]: 260.6 ± 24.08 and GAT358 [30 mg/kg]: 247.4 ± 35.86, $n = 7$ [counterbalanced]; Figure 5E). See Table S1 for individual subjects data values.

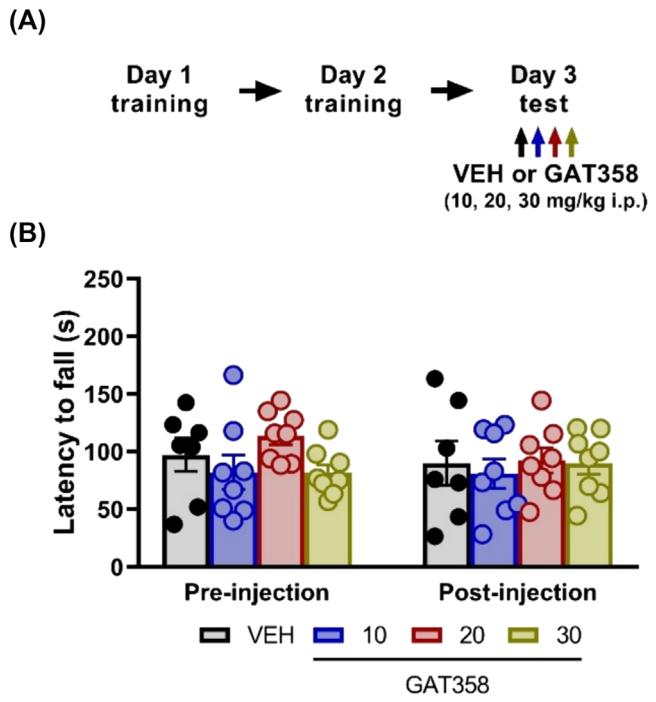

FIGURE 4 GAT358 did not produce motor ataxia in the rotarod test. (A) Schematic shows the experimental timeline. Mice were trained on the rotarod for 2 days prior being tested on the third day. Different groups were treated with vehicle (VEH, ip, black arrow) and GAT358 (10, 20 and 30 mg/kg ip, blue, red and green arrows). (B) The latency to descend from the drum did not differ between GAT358 (10, 20 or 30 mg/kg ip) or VEH treatment during either pre-injection or post-injection trials. Data are expressed as mean ± SEM, VEH $n = 7$, GAT358 10 mg/kg $n = 8$, GAT358 20 mg/kg $n = 8$, GAT358 30 mg/kg $n = 8$.

6 | DISCUSSION

CB1 NAMs have been included among the medication development priorities of the NIDA in response to the opioid crisis.[18] CB1 NAMs may represent an alternative or adjunct treatment for OUD, with the potential to produce a positive impact on society by furthering efforts to combat the opioid epidemic. It is widely recognized that OUD disrupts brain-motivated behaviours and impairs the internal control that a person has over the misused substance, effects presumably caused by changes in the reinforcing efficacy of the abused drug.[19] In the present study, we examined the impact of the CB1-biased NAM GAT358 on the reinforcing properties of morphine using a self-administration operant task in which mice received intravenous morphine infusions upon completing a specific number of nose pokes. The intravenous self-administration paradigm allowed us to evaluate the effects of GAT358 on several aspects of morphine addiction, such as drug intake, relapse or motivational strength. The results presented in this study support the hypothesis that the CB1 NAM GAT358 may be leveraged as an effective treatment to decrease morphine addictive behaviours.

GAT358, when administered (intraperitoneal) during the maintenance phase of drug self-administration (i.e., when there is an ongoing stable morphine intake), reduced the number of morphine infusions taken, as well as the number of active nose pokes and the total amount of morphine administered compared with VEH (ip) treatment. Furthermore, GAT358 reduced the escalation of morphine infusion intake and the maximum number of cumulative morphine infusions received. Moreover, when we increased the schedule of reinforcement using a PR task, GAT358 dose dependently decreased the number of infusions taken, as well as the number of active nose pokes and the motivation to work to obtain morphine infusions. Thus, GAT358 reduced both morphine consumption and motivation to self-administer the drug. These results are consistent with our previous studies that demonstrated that GAT358 decreased oxycodone consumption in a two-bottle choice paradigm, prevented CPP to morphine and eliminated morphine-induced increases in electrically evoked dopamine efflux in the mesocorticolimbic pathway.[14] To our knowledge, this is the first time that the effects of a CB1R NAM on the motivation to obtain opioids have been evaluated. Importantly, our data support the hypothesis that therapeutic interventions based on negative allosteric modulation of CB1R may reduce opioid reinforcement and motivation to self-administer opioids in models of morphine misuse.

The ability of GAT358 to reduce behaviours associated with opioid misuse was also maintained when morphine was withheld during the relapse test. GAT358 decreased the actions in the previously morphine-paired nose poke and ultimately diminished morphine-seeking behaviour to drug-cue presentations during abstinence. These observations indicate that GAT358 reduced propensity of opioid-dependent subjects to relapse following a period of abstinence. As far as we are aware, this is the only report to examine the actions of a CB1R NAM on relapse to opioids. Our findings align with previous studies demonstrating a reduction in the reinstatement of cocaine- and methamphetamine-seeking behaviour in rats employing other CB1R NAMs, such as PSNCBAM-1 and Org27569.[6,7]

Other CB1R NAMs, including PSNCBAM-1 or Org27569, have been associated with decreases in both food intake and locomotor activity, side effects that are believed to be caused by its inverse agonist profile.[20] However, it is unlikely that any of the outcomes described here with GAT358 result from nonspecific motor impairment or other adverse psychological effects. GAT358 showed a minimal CB1 inverse agonist profile and displayed functional selectivity in the β-arrestin assay.[13] In our study, GAT358 did not reduce the number of inactive nose pokes in the morphine maintenance, relapse or PR experiments, which, to some extent, lessens the possibility of nonspecific motor impairment. Nonetheless, we further assessed motor function using the rotarod test because motor ataxia hinders the execution of tasks demanding precision and coordination, like those faced in an operant task paradigm. None of the GAT358 doses employed here altered the latency to descend from the rotarod or produced motor ataxia in any instance. Furthermore, we confirmed that none of the doses of GAT358 evaluated herein reduced the motivation to work for food rewards or decreased the number of sucrose pellets consumed, consistent with absence of drug-induced anhedonia. Together, these findings suggest that GAT358 may have a more

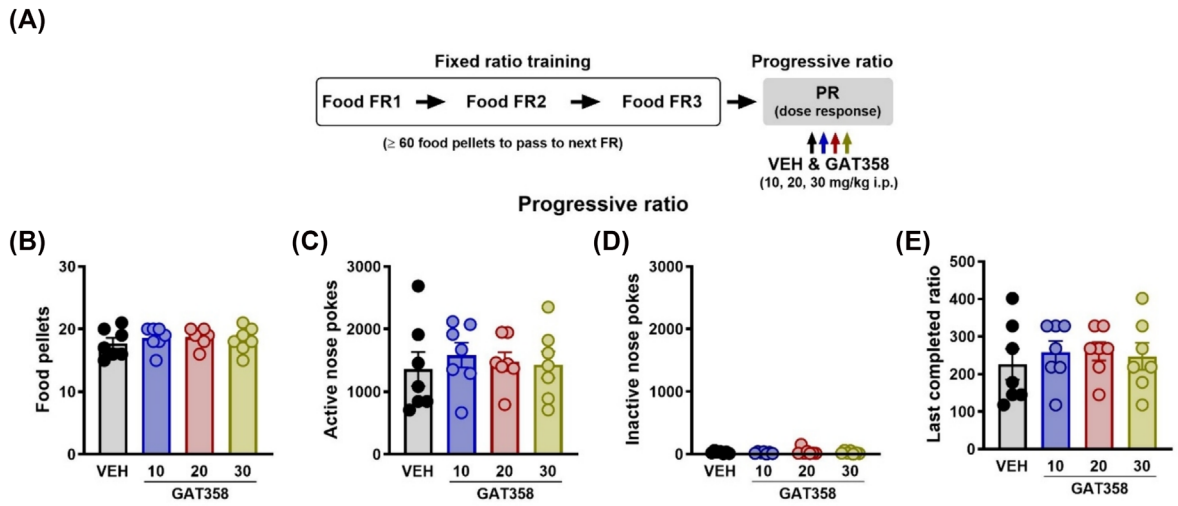

FIGURE 5 GAT358 did not affect the motivation to obtain food rewards in a progressive ratio schedule of reinforcement. (A) Schematic shows the sequence of the experimental procedures. Mice were first trained under FR1, FR2 and FR3 schedules of reinforcement with the requisite to get 60 or more food pellets to pass from one FR to the next. Then, mice underwent to PR training until they reached stable food pellet intake. After acquiring stable behaviour under the PR schedule, mice were treated in a counterbalanced manner with VEH (ip, black arrow) and GAT358 (10, 20 and 30 mg/kg ip, blue, red and green arrows) 20 min prior to behavioural testing. GAT358 did not alter the number of sucrose pellets earned (B), the number of active nose pokes (C), the number of inactive nose pokes (D) or the break point (E) during PR schedule of reinforcement at any dose. Data are expressed as mean ± SEM, $n = 7$.

beneficial pharmacological profile over other existing CB1R NAMs. More research is necessary to elucidate if the effects of GAT358 described here are dependent upon the β-arrestin-biased pharmacological profile of this ligand.

An important observation of our studies was that GAT358 not only reduced opioid-addicted behaviours when morphine was present and self-administered but also when morphine was withheld during the relapse test. As such, this observation suggests that negative allosteric modulation of CB1 cannabinoid receptor signalling influences distinct brain pathways involved in reward processing and seeking. In the VTA, endocannabinoids retrogradely modulate both glutamatergic excitatory and GABAergic inhibitory synaptic inputs onto dopamine neurons.[5] The actions of GAT358 on the VTA may account for the primary rewarding effects observed in this study. In the NAc, endocannabinoids retrogradely modulate the glutamatergic afferents from prefrontal regions onto D1-medium spiny neurons through a dopamine-independent mechanism.[21] Increased glutamate levels in the NAc are associated with cue-induced reinstatement and drug-seeking behaviours.[22–24] Hence, GAT358 could be modulating glutamatergic afferents onto the NAc during the relapse test. This hypothesis is consistent with the selective effects of disruption of protein–protein interactions downstream of the NMDARs that suppress aberrant glutamate excitability on relapse of morphine-seeking behaviour.[25] CB1R are also present in the prefrontal cortex, which processes the hedonic value and motivation to obtain drugs.[26] Thus, GAT358 could be acting in the prefrontal cortex to decrease the motivation to work for drug rewards. In our experiments, GAT358 was administered intraperitoneally, allowing for the involvement of multiple brain regions and mechanisms in mediating diverse effects of GAT358 on the reinforcing properties of morphine as described in this study. Likewise, our results do not preclude the possibility that GAT358 may differentially impact distinct neuronal populations to mediate the effects observed herein. Further research is warranted to elucidate the specific cell types impacted by GAT358 and to characterize the underlying circuit mechanisms. Future studies are also essential to determine if the results observed in male mice generalize to female mice.

In addition to the results presented here, a recent study from our lab also explored effects of the CB1R NAM GAT358 on acute antinociceptive effects of morphine, tolerance to morphine antinociception and naloxone-precipitated opioid withdrawal.[27] We showed that GAT358 did not impede morphine antinociception but was effective in reducing morphine tolerance and naloxone-precipitated opioid withdrawal.[27] As such, GAT358 may represent a promising therapeutic strategy as it produced antinociception on its own and may also potentially reduce the reliance and addiction liability of opioids. These observations raise the possibility that negative allosteric modulation of CB1R NAMs have the potential to lower the risk of opioid misuse, while exhibiting therapeutic potential for more effective pain reduction.

7 | CONCLUSION

Collectively, our findings demonstrate that GAT358 effectively decreased morphine self-administration under conditions of ongoing stable morphine consumption, lowered the motivation to work to obtain morphine infusions and reduced morphine relapse-like behaviour during forced abstinence. GAT358 did not produce side effects such as motor ataxia or anhedonia that could confound our results.

Furthermore, GAT358 also did not suppress consumption or motivation to work for food rewards. This study highlights that novel therapies based on CB1R NAMs have the potential to offer a favourable safety profile and are viable for reducing the risk associated with opioid use.

AUTHOR CONTRIBUTIONS

Idaira Oliva performed drug/food self-administration and rotarod experiments with assistance from Fezaan Kazi. Lucas N. Cantwell and Ganesh A. Thakur designed and synthesized GAT358. Idaira Oliva and Andrea G. Hohmann designed the experiments. Idaira Oliva and Jonathon D. Crystal wrote programs for data collection and analysis for the drug/food self-administration studies. Andrea G. Hohmann supervised the project. Idaira Oliva and Andrea G. Hohmann wrote the manuscript with assistance from Jonathon D. Crystal and Ganesh A. Thakur.

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CONFLICT OF INTEREST STATEMENT

G.A.T. holds a patent on allosteric modulators of CB1 cannabinoid receptors (US9926275B2). None of the other authors report any conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Chang HY, Kharrazi H, Bodycombe D, Weiner JP, Alexander GC. Healthcare costs and utilization associated with high-risk prescription opioid use: a retrospective cohort study. *BMC Med*. 2018;16(1):1-11. doi:10.1186/s12916-018-1058-y
2. Kaplan L. Medication-assisted treatment for opioid use disorder. *Nurse Pract*. 2019;44(3):10-12. doi:10.1097/00006205-900000000-99727
3. Kendall DA, Yudowski GA. Cannabinoid receptors in the central nervous system: their signaling and roles in disease. *Front Cell Neurosci*. 2017;10(January):1-10. doi:10.3389/fncel.2016.00294
4. Befort K. Interactions of the opioid and cannabinoid systems in reward: insights from knockout studies. *Front Pharmacol*. 2015;6(FEB):1-15. doi:10.3389/fphar.2015.00006
5. Maldonado R, Valverde O, Berrendero F. Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci*. 2006;29(4):225-232. doi:10.1016/j.tins.2006.01.008
6. Jing L, Qiu Y, Zhang Y, Li J-X. Effects of the cannabinoid CB1 receptor allosteric modulator ORG 27569 on reinstatement of cocaine- and methamphetamine-seeking behavior in rats. *Drug Alcohol Depend*. 2014;143(1):251-256. doi:10.1016/j.drugalcdep.2014.08.004
7. Nguyen T, German N, Decker AM, et al. Novel diarylurea based allosteric modulators of the cannabinoid CB1 receptor: evaluation of importance of 6-pyrrolidinylpyridinyl substitution. *J Med Chem*. 2017;60(17):7410-7424. doi:10.1021/acs.jmedchem.7b00707
8. Luján M, Cheer JF, Melis M. Choosing the right drug: status and future of endocannabinoid research for the prevention of drug-seeking reinstatement. *Curr Opin Pharmacol*. 2021;56:29-38. doi:10.1016/j.coph.2020.08.014
9. Horswill JG, Bali U, Shaaban S, et al. PSNCBAM-1, a novel allosteric antagonist at cannabinoid CB_1 receptors with hypophagic effects in rats. *Br J Pharmacol*. 2007;152(5):805-814. doi:10.1038/sj.bjp.0707347
10. Baillie GL, Horswill JG, Anavi-Goffer S, et al. CB_1 receptor allosteric modulators display both agonist and signaling pathway specificity. *Mol Pharmacol*. 2013;83(2):322-338. doi:10.1124/mol.112.080879
11. Cawston EE, Redmond WJ, Breen CM, Grimsey NL, Connor M, Glass M. Real-time characterization of cannabinoid receptor 1 (CB_1) allosteric modulators reveals novel mechanism of action. *Br J Pharmacol*. 2013;170(4):893-907. doi:10.1111/bph.12329
12. Straiker A, Mitjavila J, Yin D, Gibson A, Mackie K. Aiming for allosterism: evaluation of allosteric modulators of CB1 in a neuronal model. *Pharmacol Res*. 2015;99:370-376. doi:10.1016/j.phrs.2015.07.017
13. Thakur GA, Tichkule RB, Kulkarni PM, Kulkarni AR. Allosteric modulators of the cannabinoid 1 receptor. US patent 9,926,275 B2. March 27, 2018.
14. Iyer V, Rangel-Barajas C, Woodward TJ, et al. Negative allosteric modulation of CB_1 cannabinoid receptor signaling suppresses opioid-mediated reward. *Pharmacol Res*. 2022;185(August):106474. doi:10.1016/j.phrs.2022.106474
15. Sharpe AL, Varela E, Bettinger L, Beckstead MJ. Methamphetamine self-administration in mice decreases GIRK channel-mediated currents in midbrain dopamine neurons. *Int J Neuropsychopharmacol*. 2015;18(5):1-10. doi:10.1093/ijnp/pyu073
16. Kim J, Im HI, Moon C. Intravenous morphine self-administration alters accumbal microRNA profiles in the mouse brain. *Neural Regen Res*. 2018;13(1):77-85. doi:10.4103/1673-5374.224374
17. Oliva I, Donate MM, Lefner MJ, Wanat MJ. Cocaine experience abolishes the motivation suppressing effect of CRF in the ventral midbrain. *Addict Biol*. 2021;26(1):1-9. doi:10.1111/adb.12837
18. Rasmussen K, White DA, Acri JB. NIDA's medication development priorities in response to the Opioid Crisis: ten most wanted. *Neuropsychopharmacology*. 2019;44(4):657-659. doi:10.1038/s41386-018-0292-5
19. Volkow ND, Michaelides M, Baler R. The neuroscience of drug reward and addiction. *Physiol Rev*. 2019;99(4):2115-2140. doi:10.1152/physrev.00014.2018
20. Nguyen T, Thomas BF, Zhang Y. Overcoming the psychiatric side effects of the cannabinoid CB1 receptor antagonists: current approaches for therapeutics development. *Curr Top Med Chem*. 2019;19(16):1418-1435. doi:10.2174/1568026619666190708164841
21. Spanagel R. Cannabinoids and the endocannabinoid system in reward processing and addiction: from mechanisms to interventions. *Dialogues Clin Neurosci*. 2020;22(3):241-250. doi:10.31887/DCNS.2020.22.3/RSPANAGEL
22. Knackstedt LA, Kalivas PW. Glutamate and reinstatement. *Curr Opin Pharmacol*. 2009;9(1):59-64. doi:10.1016/j.coph.2008.12.003
23. Bossert JM, Gray SM, Lu L, Shaham Y. Activation of group II metabotropic glutamate receptors in the nucleus accumbens shell attenuates context-induced relapse to heroin seeking. *Neuropsychopharmacology*. 2006;31(10):2197-2209. doi:10.1038/sj.npp.1300977
24. Wenhua Zhou PWK. NAC reduces extinction responding and induces reductions in cue-and heroine-induced drug seeking. *Biol*

Psychiatry. 2008;63(3):338-340. doi:10.1016/j.biopsych.2007.06.008.N-acetylcysteine
25. Oliva I, Saberi SA, Rangel-Barajas C, et al. Inhibition of PSD95-nNOS protein–protein interactions decreases morphine reward and relapse vulnerability in rats. *Addict Biol*. 2022;27(5):1-12. doi:10.1111/adb.13220
26. Parsons LH, Hurd YL. Endocannabinoid signalling in reward and addiction. *Nat Rev Neurosci*. 2015;16(10):579-594. doi:10.1038/nrn4004
27. Iyer V, Saberi SA, Pacheco R, Sizemore EF. Negative allosteric modulation of cannabinoid CB_1 receptor signaling suppresses opioid-mediated tolerance and withdrawal without blocking opioid antinociception. *bioRxiv*. 2024;1-49. doi:10.1101/2024.01.06.574477

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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