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RESEARCH ARTICLE

Tabernanthalog Reduces Motivation for Heroin and Alcohol in a Polydrug Use Model

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Abstract

Background: The potential use of psychedelic drugs as therapeutics for neuropsychiatric disorders has been limited by their hallucinogenic properties. To overcome this limitation, we developed and characterized tabernanthalog (TBG), a novel analogue of the indole alkaloids ibogaine and 5-methoxy-*N,N*-dimethyltryptamine with reduced cardiac arrhythmogenic risk and a lack of classical psychedelic drugs-induced sensory alterations. We previously demonstrated that TBG has therapeutic efficacy in a preclinical model of opioid use disorder (OUD) in rats and in a binge model of alcohol drinking in mice. Alcohol is commonly co-used in ~35–50% of individuals with OUD, and yet, preclinical models that recapitulate this comorbidity are lacking.

Methodology: Here we employed a polydrug model of heroin and alcohol couse to screen the therapeutic efficacy of TBG on metrics of both opioid and alcohol seeking. We first exposed rats to alcohol (or control sucrose-fade solution) in the home-cage (HC), using a two-bottle binge protocol, over a period of 1 month. Rats were then split into two groups that underwent self-administration training for either intravenous heroin or oral alcohol, so that we could assess the impact of HC alcohol exposure on the self-administration of each substance separately. Thereafter, rats began self-administering both heroin and alcohol in the same sessions. Finally, we tested the effects of TBG on break points for heroin and alcohol in a progressive ratio test, where the number of lever presses required to obtain a single reward increased exponentially.

Results and Conclusion: TBG effectively reduced motivation for heroin and alcohol in this test, indicating its efficacy is preserved in animals with a history of heroin and alcohol polydrug use.

Keywords: alcohol, heroin, break point, polydrug use, tabernanthalog, self-administration

Introduction

Current treatments for opioid use disorder (OUD) are based on substituting opioid activity at the mu opioid receptor (MOR) and/or partial antagonism of the MOR to either mitigate withdrawal and/or prevent overdose in the event of relapse.[1,2] Such treatments have some of the same disadvantages as the opioids being misused, such as risk of overdose and respiratory depression.[2] The search for nonopioid-based therapeutics for OUD has led several researchers to investigate psychedelic

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drugs, many of which are thought to elicit their hallucinogenic and therapeutic effects through activation of serotonin 2A receptors (5-HT2ARs).[3–6]

Although the hallucinogenic effects of psychedelic drugs complicate their clinical use as therapeutics, the ''mystical experience'' produced by these agents is thought by many to mediate their life-transforming effects.[4,6–10] However, there is also evidence that the hallucinogenic properties of psychedelic drugs might be dissociable from their therapeutic effects.[11–15] If this is indeed the case, nonhallucinogenic analogues of psychedelic drugs could have broad potential as therapeutics.[16]

The nonhallucinogenic psychedelic drug tabernanthalog (TBG) is structurally related to ibogaine,[11] a compound with clear therapeutic application in the treatment of substance use disorders, which ultimately failed clinically due to its cardiac arrythmogenic side effects.[3] This is mediated by ibogaine's inhibitory activity at certain potassium channels,[17,18] but TBG does not possess this liability, nor does it induce classical psychedelic-like sensory alterations as measured by the head-twitch response in mice.[11] Thus, if TBG retains the therapeutic properties associated with its parent compound ibogaine, but lacks the hallucinogenic and cardiac side effects, it may be a more optimal candidate for clinical success in the treatment of substance use disorders.

Indeed, TBG has already demonstrated therapeutic efficacy in a preclinical model of OUD where rats were allowed to self-administer intravenous heroin.[11,12] TBG also reduces alcohol drinking in mice in a binge model of home-cage (HC) drinking.[11] In both these preclinical models, TBG produced long-lasting effects on heroin seeking and alcohol drinking.

Alcohol is one of the most commonly co-used substances with opioids, with ~35–50% of individuals with OUD having prior month alcohol use, often binge use,[19–21] sometimes simultaneously with opioids.[19,22] Notably, concurrent use of alcohol with opioids can increase the risk of overdose, relapse, and poor health-related outcomes.[21,23] This could render treatment of OUD with comorbid alcohol use more resistant to therapeutic outcomes and underscores the need for examining the therapeutic efficacy of potential new medications in polydrug use models.[24] Thus, to capture the complexity of polydrug use in individuals with OUD, we tested TBG in a preclinical model of heroin and alcohol polydrug use.

We first examined the impact of alcohol drinking on the subsequent self-administration of intravenous heroin or oral alcohol (separately). Then we allowed rats to self-administer both substances in the same sessions before assessing their motivation for each reward using a progressive ratio test. In this test, the price (in lever presses) required to obtain a single reward (heroin or alcohol) increases exponentially with each earned reward (separately for heroin versus alcohol levers). The maximum price paid is referred to as the animal's break point for the respective reward.[25] Before this test, rats received vehicle or TBG (30 mg/kg) injections to determine whether TBG could reduce motivation for heroin or alcohol.

Materials and Methods

Animals and surgery

All animal procedures followed guidelines approved by the University of Colorado Denver, Anschutz Medical Campus Institutional Animal Care and Use Committee. Subjects were age-matched (P50-60 on arrival) male and female Wistar rats (Charles River, Raleigh, NC, USA). Animals were always single housed in a temperature and humidity-controlled environment (lights on 8 am–8 pm) with free access to standard laboratory chow and water. All behavioral procedures were conducted during the animals' light phase.

Our procedures followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals.[26] Rats were anesthetized with ketamine/xylazine (80/7 mg/kg) and implanted with an intravenous catheter in the right jugular vein. Ketamine boosters were administered as needed to maintain anesthesia throughout the surgery. Carprofen (5 mg/kg) and cefazolin (30 mg/kg) were administered after surgery to alleviate surgical pain and prevent infection. Rats were allowed to recover from surgery before the start of the experiment (i.e., the HC drinking phase).

HC drinking phase

After recovery from surgery, rats were exposed to a 1-month period of HC drinking. Half the rats were assigned to the alcohol group, and the other half served as controls. During this phase, both groups were exposed to two bottles in the HC, in a binge-like pattern. One bottle always contained water, and the other bottle contained either alcohol or control solution (diluent for alcohol). Bottles were placed on the cages and removed from the cages during the light phase.

Rats had *ad libitum* access to water in the HC at all times. Each binge cycle lasted a total of 3 days, separated by one (binge cycle 1) to four (binge cycles 2 through 4) days. Alcohol (12%) was initially dissolved in sucrose (5%) to offset its bitter taste and promote drinking. The sucrose was gradually faded out over the first two binge cycles, such that binge cycles 3 and 4 used only autoclaved tap water as the diluent for alcohol. The position of the water bottle alternated on each binge cycle to account for any potential positional bias in drinking.

Volumes consumed were calculated based on the change in weight of the bottles from the start to the end of each binge cycle. During the last binge cycle, we

used an automated two-bottle drinking system to monitor the time course of volumes consumed over the day–night cycle. The HC drinking phase concluded with a final 4-h drinking session to assess acute volumes consumed.

Automated HC drinking

Devices for measuring HC drinking were custom built in the laboratory. The design was adapted from hackaday.io (project #162692)[27] and methods described by Godynyuk et al.[28] In brief, two 240 mL syringes were connected to sipper valves with epoxy, outfitted with eTape volumetric sensors (Milone Tech), and mounted in the HC using a 3D-printed frame. Data from the eTape sensors were collected by a Teensy 4 microcontroller and written on an SD card. Sensor data were imported into Matlab and analyzed using custom code.

Drugs and treatment protocols

Heroin (diamorphine hydrochloride; National Institute on Drug Abuse Drug Supply Program) was dissolved in 0.9% saline at a concentration of 0.8 mg/mL and filtered before use. Heroin was self-administered intravenously at a dose of 40 μg/50 μL infusion. Sucrose and ethanol were dissolved/diluted in autoclaved tap water. Ethanol (12% v/v) was initially dissolved in sucrose (5% w/v) solution, and then gradually faded out to water alone.

Alcohol was self-administered orally and delivered in a 150 μL bolus volume per reward. TBG was synthesized as described previously,[11] dissolved in sterile phosphate-buffered saline at a concentration of 10 mg/mL for dosing at 30 mg/kg (3 mL/kg, IP), and administered 30 min before testing.

Heroin and alcohol self-administration training

At the conclusion of the HC drinking phase, rats began daily self-administration training on a fixed ratio (FR1; 2.5 h/session per weekday). Half the rats from each HC drinking group (alcohol vs. control) were assigned to heroin self-administration, and the other half were assigned to alcohol self-administration. Self-administration sessions began with extension of the heroin (right) or alcohol (left) lever into the operant chamber, which was equipped with a liquid delivery port (for delivery of alcohol) in between two retractable levers. Each press on the alcohol lever delivered a single bolus of alcohol (150 μL) into the delivery port along with a tone cue (3.5 kHz, 5 s).

The lever retracted at the onset of reward delivery and for the duration of the tone cue. Each press on the heroin lever delivered an intravenous infusion of heroin (40 μg/50 μL) along with a light cue (5 s), positioned above the heroin lever. The lever retracted at the onset of infusion delivery and for the duration of the light cue. After seven FR1 training sessions, animals progressed to a variable ratio (VR) 5 schedule of reinforcement, where every fifth press (on average) on each lever resulted in delivery of the respective reward. After three VR5 sessions, rats concluded the self-administration phase on a VR15 schedule for three sessions.

To prevent infection and catheter occlusion, respectively, cefazolin and taurolidine-citrate solution were administered after each self-administration session. Catheter patency was periodically verified using sodium methohexital (0.1–0.3 cc, i.v.; 10 mg/mL in sterile water), which produces a rapid and brief loss of muscle tone in rats with patent catheters.

At the end of self-administration, rats underwent extinction training, wherein lever presses were without any consequence (i.e., no reward, no cues), resulting in the eventual extinction of drug seeking over the course of seven daily sessions. Thereafter, rats underwent a cued reinstatement session, wherein reward-related cues were available (VR5), but rewards were still withheld. Lever presses on this session served as a measure of relapse. After this, rats started co-self-administration training.

Heroin and alcohol coself-administration

After we assessed the effects of HC-drinking condition on heroin and alcohol self-administration separately, we allowed access to both substances on each lever within the same daily self-administration sessions. Importantly, because there were no statistically significant effects of prior HC-drinking condition on heroin or alcohol self-administration, extinction, or reinstatement (see Results section), these groups were pooled for the remainder of the experiment. During these co-self-administration sessions, both levers were extended simultaneously, and rewards (and their respective cues) were available on an FR3 schedule of reinforcement.

Rats were allowed to co-self-administer heroin and alcohol for five to nine sessions before assessing motivation for each reward in a progressive ratio test. Response rates for alcohol and heroin were similar and not statistically different on this low schedule of reinforcement (see Results section), and prior self-administration groups (alcohol and heroin) were pooled for the remainder of the experiment.

Progressive ratio testing

After co-self-administration of heroin and alcohol, rats underwent progressive ratio testing. On this test, both the heroin and alcohol lever were extended into the operant chamber, as during co-self-administration sessions. Rewards were available on a progressive ratio schedule of reinforcement, where the response requirement increased exponentially for each additional reward (FR1,

FR4, FR9, FR15, FR25, FR40, FR62, FR95, FR145, FR219, FR328, FR492), separately for the heroin versus alcohol lever. Levers retracted during cue delivery for each respective reward, as during all self-administration sessions.

The first progressive ratio test was conducted without any treatment to obtain a baseline assessment of differences in motivational state for heroin versus alcohol. On the second progressive ratio test, rats received an injection of TBG (30 mg/kg) or vehicle 30 min before placement in the operant chamber for testing. Rats were excluded if catheter patency was lost before testing.

Statistical analyses

All statistical tests were performed in Prism (GraphPad, 9.0). Two-way repeated measures analysis of variances (ANOVAs) with Greenhouse-Geisser correction were used to assess the effects of HC drinking on heroin and alcohol self-administration (separate ANOVAs for each reward). Break points were analyzed using two-tailed planned comparison *t*-tests for assessing the effects of TBG versus vehicle on the progressive ratio test. Significance was defined as alpha <0.05, and data are graphed as mean ± standard error of the mean (SEM).

Results

The experiment began with a 1-month period of HC drinking, wherein rats were exposed to alcohol or control (sucrose fade) solution in the HC over several binge cycles (Fig. 1a). Initially, sucrose (5%) was used to facilitate alcohol drinking, which was gradually faded to water by the third binge cycle. Custom-built devices for measuring HC drinking (Fig. 1b) were used for the final binge cycle, which allowed us to monitor volumes consumed on a second-by-second timescale.

Representative examples for cumulative consumption of alcohol and water (HC alcohol group) and water (HC control group), as well as group averages over the fourth HC consumption cycle, are shown (Fig. 1c, d). Total alcohol consumed over the entire 1-month HC drinking phase did not differ between rats assigned to subsequent heroin versus alcohol self-administration groups (Fig. 1e; mean ± SEM Heroin: 36.7 ± 6.01 g/kg; alcohol: 39.3 ± 5.73 g/kg).

To determine whether the HC drinking condition impacted subsequent self-administration of heroin or alcohol, rats that drank alcohol versus control (sucrose fade) in the HC were subsequently split into two groups that underwent either heroin or alcohol self-administration, resulting in a 2 × 2 group design: HC alcohol versus HC control drinking and heroin versus alcohol self-administration. Thereafter, rats underwent a standard self-administration, extinction, and cued reinstatement protocol for each substance (alcohol vs. heroin) to assess whether HC alcohol drinking had an impact on acquisition or intake, extinction learning, or cue reactivity under relapse conditions.

Rats were trained to self-administer heroin or alcohol on distinct levers associated with distinct cues, so that we could subsequently examine the co-self-administration of heroin and alcohol together in the next phase of the experiment. Analyses revealed only main effects of time for heroin self-administration (Fig. 2a; two-way RM-ANOVA: $F_{(2.068,28.96)} = 155.9$, $p = 2.220 \times 10^{-16}$) and alcohol self-administration (Fig. 2b; two-way RM-ANOVA: $F_{(2.200,30.79)} = 8.440$, $p = 8.879 \times 10^{-4}$), but no effect of HC drinking (control vs. alcohol) and no interaction between HC drinking and time.

The main effects of time reflect successful acquisition of heroin and alcohol self-administration, indicated by increases in the number of lever presses with the increasing response requirements for rewards over time (FR1-VR5-VR15).

After self-administration, rats successfully extinguished heroin seeking (Fig. 2a; two-way RM-ANOVA: main effect of time $F_{(1.699,23.79)} = 44.83$, $p = 2.455 \times 10^{-8}$) and alcohol seeking (Fig. 2b; two-way RM-ANOVA: main effect of time $F_{(1.774,24.84)} = 7.657$, $p = 0.003$), but there was no effect of HC drinking (control vs. alcohol) on extinction, and no interaction between HC drinking and time.

Finally, analyses comparing the last extinction day with the cue test indicated that rats showed reinstatement of drug seeking to heroin cues (Fig. 2a; two-way RM-ANOVA: main effect of time $F_{(1,14)} = 38.88$, $p = 2.180 \times 10^{-5}$) and alcohol cues (Fig. 2b; two-way RM-ANOVA: main effect of time $F_{(1,14)} = 6.620$, $p = 0.022$), but there was no effect of HC drinking (control vs. alcohol) on cued reinstatement, and no interaction between HC drinking and time. Thus, although rats in the HC alcohol group consumed a substantial amount of alcohol over the 1-month binge cycles, they did not differ compared with controls in their ability to acquire heroin or alcohol self-administration, nor in their extinction of drug seeking, or their cue reactivity during cued reinstatement.

Given the lack of effect of prior HC drinking condition on alcohol or heroin self-administration, extinction, or cued reinstatement, HC alcohol and control groups were pooled for the remainder of the experiment. Next, we allowed rats access to both the heroin lever and the alcohol lever, with both rewards available simultaneously (on an FR3 schedule) during the same behavioral sessions (Fig. 3a). At this low response requirement for rewards, the average number of lever presses for heroin versus alcohol across sessions was similar and was not statistically different.

Thus, self-administration groups (alcohol and heroin) were also pooled for the remainder of the experiment.

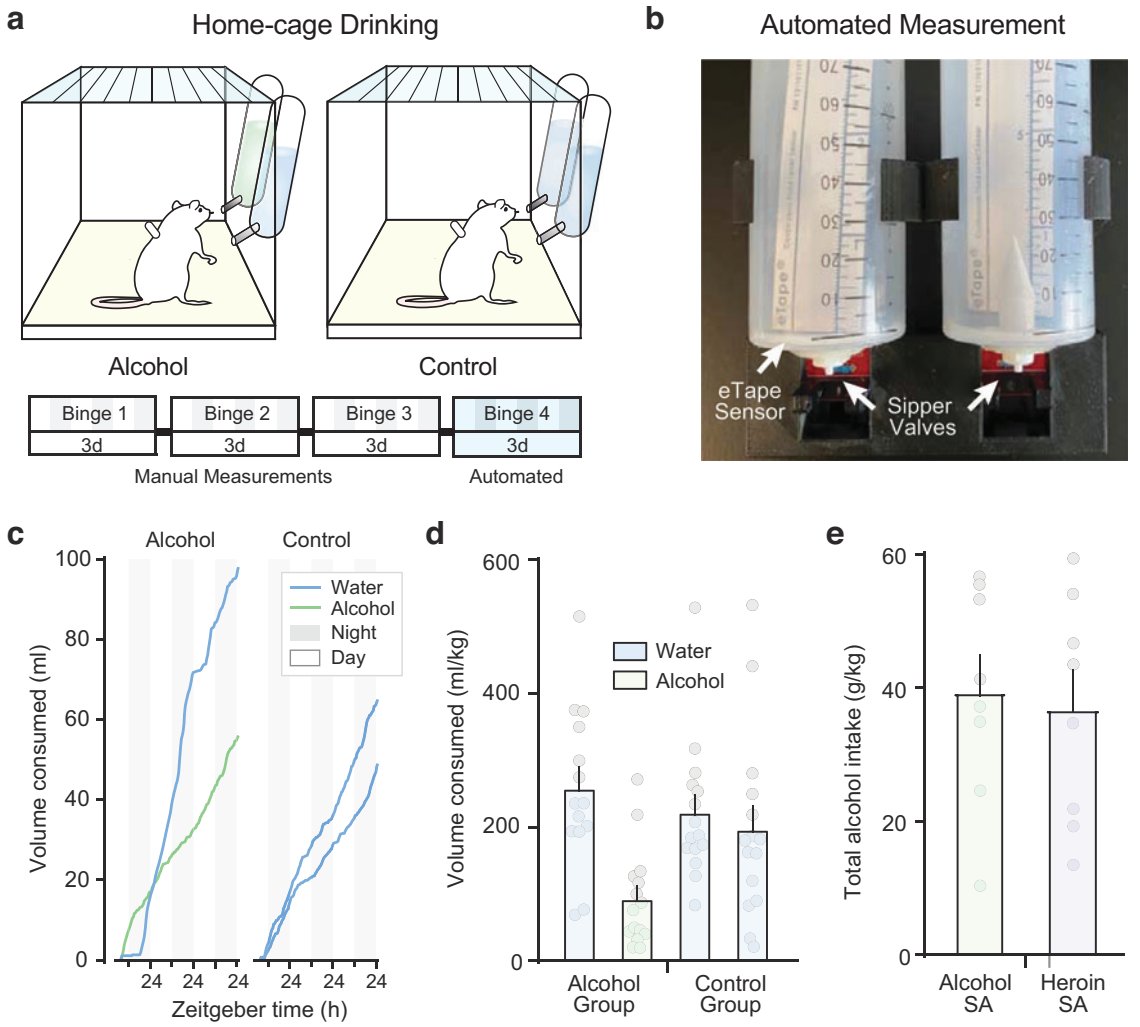

Fig. 1. HC drinking was equal for animals that subsequently self-administered heroin or alcohol. **(a)** Rats were given HC access to either alcohol and water bottles (HC alcohol group) or water bottles (HC control group) over a 1 month period during four 3-day binge cycles. **(b)** Custom HC drinking bottles containing volumetric sensors for measuring alcohol and water intake were used during the fourth and final binge cycle. **(c)** Time course showing drinking behavior for a representative HC alcohol and HC control rat during the last 3-day binge cycle. **(d)** Total volumes of alcohol and water consumed in HC alcohol ($n = 16$) and HC control rats ($n = 15$). **(e)** Alcohol drinking rats assigned to subsequent alcohol or heroin self-administration studies consumed equal amounts of alcohol (g/kg) over the entire HC drinking phase. $n = 8$ rats/group. HC, home cage.

We then tested the rats on a progressive ratio schedule of reinforcement, which assesses the animals' motivation to obtain each reward by determining the maximum price (in lever presses) each animal is willing to pay for a single reward. The price (or FR requirement) increased exponentially with each earned reward for each lever separately (heroin vs. alcohol) during the session. The last FR completed on each lever is referred to as the animal's break point for each respective reward. Results from this test revealed that break points for heroin were higher than those of alcohol (Fig. 3b; paired t-test: $t_{(25)} = 5.550$, $p = 4.523 \times 10^{-6}$), indicating that rats are more motivated for heroin.

Finally, to investigate the therapeutic efficacy of TBG on the motivational state for heroin and alcohol, rats were administered either TBG (30 mg/kg, IP) or vehicle (saline) 30 min before a final progressive ratio test. Results from this test indicated that TBG significantly reduced break points for both heroin (Fig. 4; unpaired t-test: $t_{(23)} = 2.178$, $p = 0.040$) and alcohol (unpaired t-test: $t_{(23)} = 3.783$, $p = 0.001$), planned comparisons. These results support the hypothesis that TBG retains its

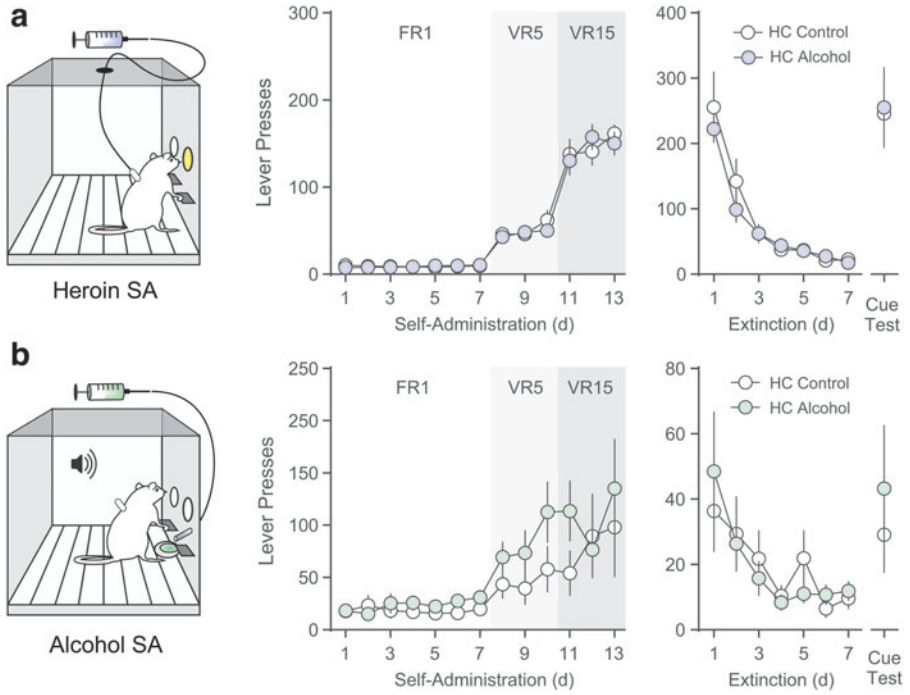

Fig. 2. HC drinking did not impact heroin or alcohol self-administration, extinction, or reinstatement. **(a)** Heroin self-administration, extinction, and cue-induced reinstatement in HC alcohol ($n = 8$) and control ($n = 8$) rats. **(b)** Alcohol self-administration, extinction, and cue-induced reinstatement in HC alcohol ($n = 8$) and control ($n = 8$) rats. There was no effect of prior HC drinking condition (alcohol or control) on subsequent self-administration, extinction, or cue-induced reinstatement for either heroin or alcohol.

therapeutic efficacy to reduce the motivation to seek heroin and alcohol in a polydrug use model where animals have a history of self-administering both substances.

Discussion

This experiment was designed to develop a model of polydrug use for heroin and alcohol, as well as to assess the therapeutic efficacy of TBG in animals with a polydrug use history. We decided to use a model that incorporated a period of prior alcohol exposure in the HC, using a two-bottle binge-drinking protocol, a commonly used procedure to habituate rats to alcohol.[29] Controls that never received alcohol in the HC allowed us to determine whether prior alcohol exposure impacted subsequent metrics of heroin versus alcohol self-administration. However, rats readily self-administered alcohol and heroin, regardless of prior HC exposure to alcohol.

Thus, going forward, the 1-month period of HC drinking could be eliminated if the goal is to examine outcome measures in an operant self-administration model. However, we caution that this model should not be considered a model of alcohol use disorder, but rather reflects repeated low dose (i.e., recreational) alcohol consumption. Chronic intermittent ethanol exposure or binge-like drinking paradigms are more effective at inducing alcohol dependence or high alcohol intake if the primary goal is to model alcohol use disorder.[30,31]

After self-administration of alcohol or heroin, rats were allowed to self-administer both substances (on opposing levers, with distinct cues associated with each reward). On a low-effort response requirement (e.g., FR3), responding for alcohol and heroin was similar during the co-self-administration phase. Alcohol is known to be a low-to-moderate reinforcer in rodents,[32] perhaps owing to the slow pharmacokinetics of an orally self-administered substance and/or the bitter taste, which is why sweet tastants (e.g., sucrose/saccharin) are sometimes used to enhance alcohol taking, similar to the sucrose-fade procedure we used during the HC drinking phase.[33]

However, once the sucrose was faded out by the third binge cycle of the HC drinking phase, water was used as the diluent for alcohol for the remainder of HC drinking and for alcohol self-administration. Thus, Wistar rats will readily self-administer alcohol (12% v/v) without the use of sweet tastants. Indeed, Wistar rats have been suggested to be the preferred strain for addiction research given their proclivity to escalate their intake of alcohol and other substances.[34–36]

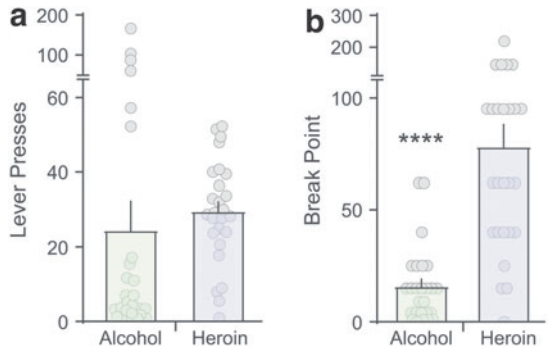

Fig. 3. Motivation for heroin is higher than that for alcohol. **(a)** Rats co-self-administration heroin and alcohol at equal rates when both rewards were simultaneously available on an FR3 schedule of reinforcement. **(b)** Break points measured during a progressive ratio test of motivation revealed significantly higher break points for heroin than for alcohol. $n = 26$ rats. ****$p < 0.0001$.

We knew from our prior study that rats are highly motivated for heroin, by several orders of magnitude higher than they are motivated for food.[37,38] This finding was a bit surprising to us given that food is a necessity for survival, whereas heroin might be considered a ''luxury'' reward.[38]

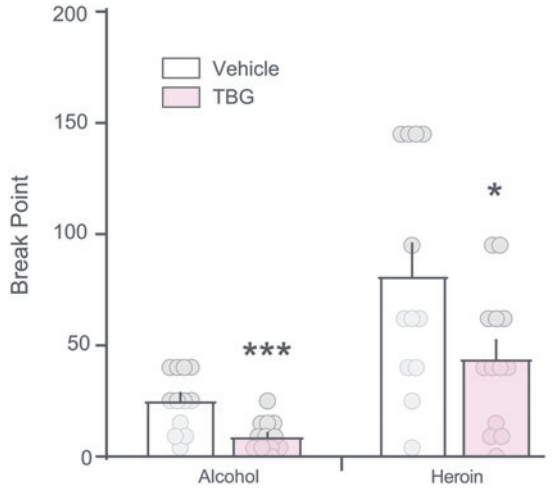

Fig. 4. TBG reduces motivation for both heroin and alcohol. TBG (30 mg/kg; $n = 13$ rats) or vehicle (saline; $n = 12$ rats) was administered 30 min before testing. TBG significantly reduced both heroin and alcohol break points. *$p < 0.05$, ***$p < 0.001$ compared with vehicle. TBG, tabernanthalog.

However, it should be noted that food was available *ad libitum* in the HC throughout these experiments, as food deprivation is known to be a stressor that reinstates heroin seeking,[39] and we did not want this to be a potential confounding variable in our experiments.

We focused on break points for heroin and alcohol assessed using a progressive ratio test, as we have previously shown the motivation for heroin to be higher than food in this assay.[12] In our previous study, we reported that the same dose of TBG (30 mg/kg) reduced heroin, but not food, break points. Interestingly, alcohol break points in this study were similar in magnitude to those for food in our other study.[12] Thus, it is unlikely that the lack of effect on motivation for food was mediated by a floor in break point measurements, adding confidence to our previous conclusion that TBG does not impact food motivation.[12] Coupled with observations from this study, this suggests that TBG selectively reduces motivation for drug but not food reward.

We are only beginning to understand the neural mechanisms through which classic psychedelic drugs and their nonhallucinogenic analogues produce therapeutic effects, but the neuroplasticity-promoting properties of these agents are likely crucial for long-term benefits.[40] Indeed, psychedelic drugs, nonhallucinogenic psychedelic-derived compounds such as TBG, and nonpsychedelic compounds such as ketamine all acutely promote structural plasticity in prefrontal cortical neurons.[41–45]

Similar plasticity promoting effects are observed acutely in the cortex after the administration of a monoamine oxidase inhibitor, or after chronic treatment with a selective serotonin reuptake inhibitor.[46,47] Intriguingly, the plasticity-promoting effects of psychedelic drugs might be dissociable from their hallucinogenic properties.[48] Although some pharmacological studies have suggested that activation of the 5-HT2A receptor may not be necessary for psychedelic drug-induced neuroplasticity,[14,42] others have used genetic knockout animals to demonstrate a critical role for 5-HT2AR in this process.[49,50] Nonetheless, the plasticity-promoting effects of these compounds *in vivo* reported to date require more time (i.e., >6 h) than the pretreatment time used for TBG (30 min) in our current study.[43]

Thus, although the long-term therapeutic effects of TBG and similar agents on motivation for drugs of abuse depend on their plasticity promoting properties,[11,12] intracellular signaling pathways triggered by acute receptor activation are likely responsible for the acute effects of TBG on the motivation for alcohol and heroin in this study.

Opioid and alcohol use produce complex changes in the serotonin system, and the potentially compounding effect of their couse has not been investigated to date.[51] Exposure to either substance has been linked to an increase in the expression of cortical 5HT2 receptors[52,53]

that may be compensatory given that 5-HT2A agonists reduce motivation for alcohol and opioids.[54–57] Supporting this notion, loss of function single nucleotide polymorphisms in the human *htr2a* gene have been associated with an increased severity of both alcohol and heroin use.[58]

One of these mutations, the T102C CC polymorphism, reduces the expression of 5-HT2A receptors in the cortex, and is associated with impaired impulse control and an increased risk of relapse in patients with alcohol use disorder.[59–61] These findings suggest that the alcohol and heroin motivation-reducing effects of TBG that we observed in our study might be mediated by its activation of the 5-HT2A receptor.

The pharmacology of TBG is, however, complex as it targets multiple receptor systems involved in the modulation of opioid reward and opioid withdrawal, including 5-HT2A/C, 5-HT1B, alpha2 adrenoreceptors, the serotonin transporter, and monoamine oxidase.[11] A complex interplay between the 5-HT2A and 5-HT2C receptor in the prefrontal cortex has been associated with the regulation of cognitive functioning, which may involve a physical interaction at the cell membrane.[62–65]

Although the 5-HT2A receptor is likely an important target for TBG's therapeutic effects, the precise brain locus, and mechanisms through which TBG acts to reduce heroin and alcohol motivation have yet to be examined in detail. Thus, additional future research is warranted to elucidate the neurobiological and potential polypharmacological mechanisms through which TBG confers its therapeutic effects.

Conclusions

Here we report that TBG effectively reduces motivation for heroin and alcohol in a polysubstance use model. Our results add to the growing evidence for TBG as an effective treatment for substance use disorders and other neuropsychiatric conditions.[11,12,66] We have extended this evidence to show that the therapeutic efficacy of TBG is maintained in animals with a history of polydrug heroin and alcohol co-use. As alcohol drinking and self-administration in this study resulted in moderate amounts of alcohol consumption, we consider this a model of OUD with comorbid recurrent alcohol use, not rising to the level of full-blown alcohol use disorder.

As a large proportion of individuals with OUD regularly use alcohol as well, we believe our findings have translational relevance for this population. Thus, our findings suggest that TBG holds therapeutic promise for reducing both heroin and alcohol motivation in animals with a history of polydrug use of both substances. Clinical trials will be necessary to confirm TBG's safety and reduced side effect profile, as well as its efficacy in OUD patients, including those with regular couse of alcohol.

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Authors' Contributions

J.A.H., G.G., and J.P. designed the study. J.B., G.G., and J.P. performed experiments. J.A.H., G.G., and J.P. analyzed data. J.A.H. and J.P. wrote the article with input from all authors.

Author Disclosure Statement

J.P. is a consultant for Delix Therapeutics, Inc. D.E.O. is a cofounder of Delix Therapeutics, Inc., serves as the chief innovation officer and head of the Scientific Advisory Board, and has sponsored research agreements with Delix Therapeutics.

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