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Preclinical assessment of the abuse potential of purified botanical cannabidiol: self-administration, drug discrimination, and physical dependence

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Running Title: Abuse potential of botanical cannabidiol

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ABSTRACT

Cannabidiol (CBD) is a constituent of the cannabis plant with a diverse array of pharmacological activities as well as potential therapeutic uses. An oral formulation of CBD (Epidiolex® in the US; Epidyolex® in Europe) is approved for treating seizures associated with rare and severe forms of epilepsy. These studies, which supported the approval of the medication, investigated abuse-related effects of CBD in rats and nonhuman primates (NHPs) using drug self-administration, drug discrimination, and physical dependence procedures and characterized its pharmacokinetics. In NHPs (n=5) that self-administered midazolam (0.01 or 0.032 mg/kg/infusion), CBD (0.1-3.2 mg/kg/infusion) failed to maintain responding above vehicle levels. CBD maintained very modest levels of self-administration in rats (n=7-8) that self-administered heroin (0.015 mg/kg/infusion) and did not increase drug-lever responding, up to a dose of 150 mg/kg (p.o.), in rats (n=6) trained to discriminate 0.5 mg/kg (i.p.) midazolam. In juvenile (5-6 weeks old) and adult (10-11 weeks old) male and female rats, discontinuation of chronic treatment (twice daily for 20 days) with an oral formulation of CBD (20 or 100 mg/kg, p.o.) did not reliably produce signs of withdrawal. Pharmacokinetic studies confirmed that the dosing regimens used in these studies resulted in therapeutically relevant plasma levels. Taken together, the lack of reliable self-administration, the failure to increase drug-lever responding in rats trained to discriminate midazolam, and the absence of withdrawal signs upon discontinuation of chronic treatment indicate that CBD has very low abuse potential and is unlikely to produce physical dependence.

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Significance Statement

Legalization of cannabis across the United States and elsewhere has led to intense investigation into the safety and therapeutic potential of cannabis and its constituent materials, including cannabidiol (CBD). Results of these preclinical abuse potential studies on CBD indicate no rewarding properties, physical dependence potential, or similarity to a benzodiazepine~~or THC~~. Together with data from *in vitro* pharmacology and human abuse potential studies, the abuse potential of Epidiolex in humans is likely to be negligible.

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INTRODUCTION

Increased legalization of cannabis for medicinal and/or recreational use across the United States, Canada, and other territories over the last decade has led to intense investigation into the safety and therapeutic potential of cannabis and its constituent materials. Cannabidiol (CBD) is a constituent of the cannabis plant with a diverse pharmacology, engaging multiple molecular targets such as 7-transmembrane receptors (e.g., G protein-coupled receptors), metabotropic and voltage-gated ion channels, transporter enzymes, and several intracellular targets (e.g., Ibeas Bih et al., 2015). Many of these targets have been implicated in a range of medical conditions prompting substantial interest in its therapeutic potential (Devinsky et al., 2014; Britch et al., 2021). Importantly, CBD also appears to have a favorable safety profile, notably, having very low affinity for and no pharmacological efficacy at CB1 cannabinoid receptors (Pertwee 2008), which is the primary site of action for delta-9-tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis.

Until recently, approved cannabis-derived medications included synthetic THC (Dronabinol and Nabilone), used for the treatment of nausea and vomiting associated with cancer treatments, and a 50:50 mixture of botanical CBD and THC (Sativex®), used in territories outside of the US for the treatment of spasticity associated with multiple sclerosis. In 2018, based upon the demonstration of efficacy in three Phase III clinical trials, the US Food and Drug Administration (FDA) approved an oral solution containing CBD (Epidiolex) for treating seizures associated with two rare, severe forms of epilepsy, Dravet Syndrome and Lennox Gastaut Syndrome (FDA 2018). As such, Epidiolex became the first FDA-approved drug to contain a purified extract from the cannabis plant. The US Drug Enforcement Administration (DEA) soon after placed Epidiolex in Schedule V, reflecting the negligible

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potential of abuse associated with therapeutic dosing and low probability of illicit misuse (DEA 2018).

This paper describes *in vivo* behavioral pharmacology and pharmacokinetic studies investigating abuse-related effects of highly purified (<0.1% THC) CBD using the Epidiolex active pharmaceutical ingredient (API), highly purified botanical CBD of clinical grade, generated in accordance with Good Horticultural Practice and Good Manufacturing Practice. Conducted in parallel with *in vitro* studies examining affinity for and functional activity at molecular targets implicated in abuse potential (e.g. *mu* opioid receptors, monoamine transporters) as well as human studies in subjects with a history of drug abuse, the current studies evaluated the following: 1) positive reinforcing effects of CBD in rats and NHPs (rhesus monkeys) trained to self-administer the μ-opioid receptor agonist heroin or the benzodiazepine midazolam, respectively; 2) discriminative stimulus effects of CBD in rats trained to discriminate midazolam; and 3) physical dependence and withdrawal in rats treated daily with CBD. Dose-finding studies (i.e., effects of pretreatments on food-maintained operant behavior) evaluated behavioral activity of test doses and bioanalytical (pharmacokinetic) studies were conducted to ensure test doses produced clinically relevant plasma concentrations.

CBD has no affinity for CB1 or CB2 receptors (Pertwee, 2008) and numerous studies have shown that CBD does not have psychotropic effects in humans although it can produce somnolence. The regulatory agency (FDA) position was that CBD could have some sedative and/or euphoric properties, thus warranting comparison to benzodiazepines or barbiturates. In regulatory abuse/dependence experiments, positive controls must be controlled substances, hence the decision to use benzodiazepines as comparators in the drug-discrimination and self-administration experiments. Although nonhuman primates readily self-administer benzodiazepines, these drugs generally are not self-administered by rats. Rats first need to be

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trained to self-administer a drug with robust positive reinforcing effects, such as heroin. As shown in these studies, after acquisition of heroin self-administration followed by saline extinction, rats will self-administer benzodiazepines like diazepam and midazolam at levels greater than saline.

MATERIALS AND METHODS

Animal use approvals

Studies were performed in accordance with current laws and guidelines at the sites where experiments were conducted. NHP studies were performed in the United States according to guidelines of the Institutional Animal Care and Use Committee, the University of Texas Health Science Center at San Antonio, and the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (2011). Rat self-administration and drug-discrimination experiments were performed in the United Kingdom in strict accordance with Home Office (United Kingdom) Guidelines and licensed under the Animals (Scientific Procedures) Act 1986. Rat studies on physical dependence were performed in France in accordance with Council Directive No. 2010/63/UE (2010) on the protection of animals used for scientific purposes, French decree No. 2013-118 (2013) on the protection of animals, and as recommended by Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Test and control substances

All studies with CBD used the purified botanical Epidiolex API containing ≥ 98% CBD and ≤ 0.1% THC in addition to other cannabinoids (w/w). In other studies, this API was used to formulate material for administration in humans and in clinical studies designed to assess

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safety in healthy volunteers as well as efficacy and safety in patients (Devinsky et al. 2016, 2017, 2018; Schoedel et al. 2018; Thiele et al. 2018). CBD solutions were freshly prepared on the day of the experiment.

For i.v. self-administration studies in NHPs, CBD was dissolved in a mixture of 9.1% ethanol, 9.1% emulphor, and 81.8% sterile saline (v/v/v; 1:1:9 vehicle) and midazolam hydrochloride (West-Ward Pharmaceuticals Corp., Eatontown, NJ, USA) was purchased as a solution (5 mg/ml in saline) and diluted to the appropriate concentrations in the 1:1:9 vehicle. For i.v. self-administration studies in rats, CBD was dissolved in a vehicle comprising 5% (w/v) Solutol HS15 (Kolliphor HS15, Sigma-Aldrich Company Ltd., Dorset, England, UK) in 0.9% (w/v) saline (Baxter Healthcare Corp. Berkshire, England, UK) and protected from light with silver foil. For rat studies, samples of each CBD dosing solution were taken for concentration analysis. All samples were all within ±12.5% of the theoretical compound levels. Heroin hydrochloride (Johnson Matthey, MacFarlan-Smith, UK) was dissolved in sterile 0.9% (w/v) saline (Baxter Healthcare Corp. Berkshire, England, UK) whereas midazolam hydrochloride (Hypnovel®; Roche Products Limited, Hertfordshire, England, UK) and diazepam injection ampules (5 mg diazepam base/ml; Hameln Pharma Ltd. Gloucestershire, UK) were purchased as a solution; midazolam was diluted with sterile 0.9% (w/v) saline (Baxter Healthcare Corp. Berkshire, England, UK) and diazepam was diluted with 2% Tween 20 in sterile 0.9% (w/v) saline (Baxter Healthcare Corp. Berkshire, England, UK).

For drug discrimination and physical dependence studies in rats, drugs were administered p.o. The formulation of CBD used in these studies was the same as the oral formulation used for clinical studies in humans (CBD-OS); a solution containing 100 mg/ml CBD was diluted in a vehicle (placebo oral formulation) containing ethanol (79 mg/ml), sucralose (0.5 mg/ml), strawberry flavoring (0.2 mg/ml), sesame oil (q.s. to 1 ml) with a final

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solution containing 10% (v/v) ethanol. Two CBD dose formulation samples from each dose tested in the drug discrimination studies were collected for concentration analysis. Samples were within ±10% of the nominated values. Midazolam (Hypnovel®; Roche Products Limited, Hertfordshire, England, UK) was diluted in deionized water. Alprazolam was suspended in 0.25% (w/v) methylcellulose in deionized water for oral dosing. Dose volumes were 2 ml/kg for p.o. dosing for CBD and 5 ml/kg midazolam and alprazolam. Oral doses were given by gavage. Diazepam (Cooperation Pharmaceutique Française, Melun France) was dissolved in 0.2% hydroxypropylmethylcellulose (Sigma-Aldrich Chimie S.a.r.l., Lyon, France) in distilled water. Morphine hydrochloride (Caesar Loretz GmbH, Bonn, Germany) was dissolved in distilled water.

Dose-finding and self-administration studies in NHPs

Subjects. Six adult NHPs (three male and three female) were individually housed and maintained on a 14 h/10 h light/dark cycle, at a temperature of 21 ± 1° C, and at a relative humidity of 50 ± 10%. One NHP was used for the dose-finding study only, one for dose finding and self-administration, and four for self-administration only; all had experience with various drugs in previous studies. NHPs were allowed free access to water while in the home cage and received primate chow (High Protein Monkey Diet; Harlan Teklad, Madison, WI, USA) and other appropriate sustenance, which maintained age-appropriate body weights. Environmental enrichment, such as foraging devices, were provided several times per week.

Surgery. NHPs were instrumented with a chronic s.c. access port (MIDA-PU-C50; Instech Laboratories, Plymouth Meeting, PA, USA) and an indwelling i.v. catheter (e.g., jugular or femoral vein). For port and catheter implantation, NHPs were initially anesthetized with ketamine (10 mg/kg i.m.) then maintained on isoflurane (1.5-3%). Surgery was conducted by a veterinarian under sterile conditions. Catheter positioning was confirmed with a fluoroscope;

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the catheter was tunneled from the site of implantation to the back where the access port was implanted s.c. After surgery, NHPs were allowed at least 4 days for recovery and received an injectable antibiotic and an analgesic (meloxicam, 0.2 mg/kg the first day then 0.1 mg/kg per day for 3 to 4 days as needed).

Apparatus. During dose-finding and self-administration sessions, NHPs were seated in chairs (model R001; Primate Products, Miami, FL, USA), which were placed in ventilated, sound-attenuating chambers containing two response levers and associated stimulus lights. Only one of the two levers was active for any individual NHP. For the dose-finding study, a food hopper located outside the chamber delivered food pellets to a receptacle centrally located on the panel below the levers. For the self-administration study, an infusion pump (model PHM-100; MED Associates, Inc., St. Albans, VT, USA) located outside the chamber was connected to the catheter with sterile tubing and a Huber point needle. The response panels, pellet dispensers, and infusion pumps were connected to and controlled by an interface and computerized system (MED Associates, Inc., St. Albans, VT, USA).

Dose-finding study. In advance of the self-administration study, an i.v. dose-finding study was conducted in two NHPs (one female and one male) to determine the upper dose limit of CBD to be examined in the self-administration study. Sessions consisted of 8 cycles comprising a 10-minute timeout, during which the chamber was dark and lever presses had no programmed consequence, followed by a response period during which the light located directly above the active lever was illuminated green and food pellets (300 mg raspberry sucrose pellets) were delivered according to a fixed ratio (FR) 10 schedule. The chamber was darkened after the delivery of 10 food pellets or 5 minutes, whichever occurred first. Responding was considered stable when mean response rates for each session, obtained by averaging rates across cycles, were within ±20% of the mean of 5 consecutive sessions. Tests

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were conducted every third session as long as that criterion was satisfied during the intervening training sessions. If that criterion was not met between tests, additional training sessions were conducted until testing criteria were satisfied for 2 consecutive sessions. Thereafter, vehicle or CBD (1.0, 3.2, and 5.6 mg/kg) was administered i.v. immediately before the first cycle of test sessions. NHPs were tested twice with vehicle (once before and once after tests with CBD) and once with each dose of CBD. Only two NHPs participated in this experiment; therefore, no additional statistical analysis was performed.

Self-administration. Reinforcing effects of CBD were examined in five NHPs trained to self-administer midazolam using standard procedures as described previously (e.g., Maguire et al. 2020). Immediately prior to each session, NHPs received a non-contingent infusion of the unit dose that was available for self-administration during the session. At the beginning of the session, the green light over the active lever was illuminated and 30 consecutive responses on the active lever (FR 30) turned the green light off, turned the red light on for 5 sec, delivered an i.v. infusion, and initiated a 180-sec timeout. During the timeout, all stimulus lights were off and responding had no programmed consequence. Responses on the inactive lever reset the response counter on the active lever but otherwise had no programmed consequence. Sessions lasted 90 min, inclusive of response periods and timeouts. Initially, NHPs responded for i.v. infusions of midazolam (0.032 mg/kg/infusion in two NHPs and 0.01 mg/kg/infusion in three NHPs) in 1:1:9 vehicle. A minimum of 5 midazolam self-administration sessions were conducted to establish stability of performance, as defined by 3 consecutive sessions in which NHPs received at least 6 infusions per session with less than 20% variance in mean number of infusions across those three sessions. Thereafter, vehicle replaced midazolam for a minimum of 4 sessions and until the following extinction criteria were met: 1) the number of vehicle infusions obtained in each of 3 consecutive sessions was less than half the average number of

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midazolam infusions obtained; and 2) average response rate across those same 3 sessions was less than 20% of the average response rate across the last 3 sessions when midazolam was available. Next, two doses of CBD (first 0.1 and then 0.32 mg/kg/infusion) were studied, each followed by vehicle extinction, and then by reassessment of midazolam followed again by vehicle extinction. Then, two additional doses of CBD (first 1.0 and then 3.2 mg/kg/infusion) were studied, each followed by vehicle extinction, and then a final assessment of midazolam. Each dose of CBD was studied for a minimum of 5 and a maximum of 10 consecutive sessions. The number of responses made on each lever during response and timeout periods, as well as the number of infusions received, was recorded for each session. The number of infusions obtained for each session was the primary dependent measure. The number of infusions obtained across the last 3 sessions of each condition was averaged for each individual NHPs, and these data were analyzed using a one-way repeated measures ANOVA with Geisser-Greenhouse correction followed by Dunnett's post-hoc comparison (GraphPad Prism, version 9.1.2, GraphPad Software, LLC, San Diego, CA, USA).

Drug discrimination studies in rats

Subjects. Female Lister Hooded rats were purchased from Charles River (Kent, England, UK) and arrived in the laboratory when they were 4 weeks old. Rats were housed in groups of 4 in solid-bottom cages. Upon arrival, rats were acclimated for 6-7 days before the study began. The animal rooms were illuminated by fluorescent lights timed to give a 12 hr/12 hr light/dark cycle (on 07.00 hr; off 19.00 hr) at a temperature of 21 ± 4°C and relative humidity of 55 ± 20%. Rats were allowed free access to a diet of Harlan Teklad Global 18 Rodent Diet (Envigo, Huntingdon, UK). Drinking water was available *ad libitum* to all animals except when they were in the testing boxes.

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Apparatus. The studies were conducted using commercially available (MED Associates, Inc., St. Albans, Vermont, USA) operant chambers measuring 30.5 x 24.1 x 21.0 cm high, located within sound attenuating, ventilated cubicles (Model ENV-018MD; MED Associated, Inc.). Each chamber was equipped with two levers located 11.5 cm apart. The chamber was fitted with a house light and a 5 x 5 cm opening located equidistant between the levers for food pellet/milk delivery. Data were collected and stored by a microprocessor and associated interface (MED PC Version 5, MED Associates, Inc.). Each chamber was equipped with an infrared camera (Model 170IR, RF concepts) which relayed images to a digital video recorder (Model RF2421, RF Concepts) and were displayed on a computer monitor. The cameras allowed the operator to monitor animal behavior inside the chambers.

Drug discrimination. Initially, a response on either lever resulted in delivery of sweetened milk. The response requirement was gradually increased from 1 to 5 (FR 5) responses before discrimination training began. Pharmacokinetic analysis revealed a Tmax of 120 min after oral administration of CBD to rats. To ensure that doses of CBD (20, 75 and 150 mg/kg p.o.) would not impair responding, its effects were investigated in animals that responded under the FR 5 schedule. Groups of 5 rats received CBD 120 min before 10-min sessions. Any effects of CBD on general behavior of the rats were also noted. These animals were included in the subsequent drug discrimination study.

Rats received an i.p. injection of midazolam (starting at 0.2 mg/kg and gradually increasing to 0.5 mg/kg) or vehicle (saline; 1 ml/kg) after which they were returned to their home cage; 15 min later, they were placed in operant condition chambers for sessions lasting 10 min. During sessions, five responses on the lever designated correct by the injection given before the session resulted in the delivery of sweetened milk whereas responding on the other lever had no programmed consequence. Following a saline injection, the contingencies were

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reversed. The lever designated correct after an injection of midazolam was assigned randomly across rats and remained constant for all training sessions. When rats achieved approximately 60% correct responses during most sessions, testing commenced.

Treatments given during testing were alternated to prevent rats from learning a particular sequence. Training sessions between tests were randomized (e.g. SR MT MR ST) with each symbol representing drug treatment on a single day: S = saline; M = midazolam; R = reinforcement day (reinforcers were delivered each time rats completed the response requirement on the correct lever for the entire 10 min session); and T= test day (reinforcers were delivered each time rats completed the FR5 response requirement on either lever in the 10 min session; responses on a lever did not have to be consecutive). In a previous drug-discrimination study at RenaSci Ltd, the lack of food rewards in the first 2.5 min of the session produced lever switching and the criterion for percentage of correct responses on the midazolam and saline levers was not attained. This was not due to inability to discriminate between midazolam and saline because when responding on the correct lever was rewarded during training sessions, the majority of rats responded >80% on the correct lever in every session. In addition, performance of rats in the final 7.5 min of the test session when responding on both levers was rewarded (i.e., there is no bias for the rats to select either one lever or the other) was much better than in the initial 2.5 min non-rewarded part of the test. The criterion for acceptable performance during ~~testing~~ training was ≥75% of responses emitted on the injection-appropriate lever during the session preceding a drug test and a mean of ≥75% responding on the injection-appropriate lever in 4 consecutive sessions in which rats received midazolam and 4 consecutive sessions in which they received saline, after which they progressed to other drugs.

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CBD and alprazolam were assessed in the same manner with the percentage of responses on the midazolam lever determined during the 10-min session. Rats had to complete correctly one saline and one midazolam test or reinforcement session in a random order between each compound test (CT), typically as follows: ST MR CT MT SR CT. Saline and midazolam sessions were repeated if a rat showed unacceptable performance. Rats were tested once per day, 4-5 days per week, with a 'washout' period of at least 24 hr between drug treatments. Prior to each rat being placed in a chamber, the levers and walls were swabbed with 10% ethanol solution to prevent olfactory stimuli from the previous rat influencing responding of the subsequent rat (Extance and Goudie, 1981). CBD and alprazolam were given by gavage before sessions. The dose test intervals were equivalent to the Tmax for each drug. CBD was tested at 3 doses given 120 min before sessions and alprazolam was tested at 4 doses given 30 min before sessions. Testing criteria for drug discrimination studies were as follows: full generalization = ≥75% responses on the midazolam lever; partial generalization = 25.1 - 74.9% responses on the midazolam lever; and no generalization = ≤25% responses on the midazolam lever.

During some test sessions, responding was markedly suppressed by test compounds (i.e., number of responses in the 10-min session was decreased to ≤50% compared with the mean number of responses in the previous 4 sessions made by the same rat when tested with the training drug); drugs producing this effect were considered to disrupt behavior. In these cases, the test session was repeated the following testing day. If responding improved to acceptable levels, this result was considered valid. If responding continued to be suppressed, the result for the rat was recorded as disrupted responding. When a dose produced at least a 50% decrease in responding in at least 50% of the rats, it was classified as "behavioral

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disruption" and larger doses were not tested. Statistical analysis was not performed for the drug discrimination study.

Dose-finding and self-administration studies in rats

Subjects. Male Sprague-Dawley rats (200–275 g) were purchased from Charles River (Kent, England, UK) and housed individually in plastic cages on a 12 h/12 h light/dark cycle at a temperature of 21 ± 4° C and at a relative humidity of 55 ± 20%. Rats were allowed free access to food (Harlan Teklad Global 18 Rodent Diet, Envigo, Huntingdon, UK) during the acclimatization period, and then they were restricted to 10 g food/day for 5 days. At the start of this 5-day food restriction, lever press training commenced. After this time, daily food intake was restricted to ~90% of normal levels (mean daily food intake during acclimatization), sufficient to maintain age-appropriate growth. Body weights were monitored daily throughout the study and the amount of food given in home cages was adjusted when necessary. This regime was maintained throughout the remainder of the study, except for 24 hr before surgery and for 48 hr after surgery, during which time animals had free access to food. Drinking water was available ad libitum except when rats were in the testing boxes.

Surgery. Chronic indwelling i.v. catheters were implanted into the right jugular vein under isoflurane anesthesia (2.25 - 2.5% in O_2, 1 liter/min). Carprofen (5 mg/kg, s.c.) was given immediately before surgery. The sterile catheter (IVSAp40 silicone tubing; Camcaths Ltd, UK) was tunneled s.c. from the site of insertion to the midscapular region where it was attached to an access port. Rats received sterile saline (1 ml/kg, i.v.) at the end of surgery. Antibiotic drugs were administered prophylactically: enrofloxacin (Baytril; 5 mg/kg/day, s.c.) on the day of surgery and 24 hr later and ticarcillin/calvulonate (Timentin; 80 mg/kg/day, i.v.) daily for the remainder of the study. Beginning 48 hr after surgery, catheters were flushed daily with a volume of heparinized saline (30 iU/ml) equal to the volume of the IVSAp40 access port and

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catheter. Catheter patency was confirmed daily by gently drawing back and observing freely flowing blood in the catheter line. If catheter patency failed during the study, as detected by visible evidence of leakage, occlusion of the tubing, or failure of rats to show immediate sedation upon i.v. injection of propofol (1.625 mg/kg), the rat was euthanized by a U.K. Home Office Schedule 1 procedure.

Apparatus. Experiments were conducted in commercially available operant conditioning chambers measuring 30.5 x 24.1 x 21.0 cm and located within sound-attenuating, ventilated cubicles (Med Associates, Inc., St. Albans, Vermont, USA). Each chamber was equipped with two levers positioned 11.5 cm apart with 2.5 cm translucent stimulus lights above each lever. A 5 x 5 cm opening was located equidistant between levers; food pellets could be delivered to the opening from a food hopper mounted outside of the chamber. The response panels, pellet dispensers, and infusion pumps were connected to and controlled by an interface and computerized system (MED Associates, Inc., St. Albans, VT, USA).

Dose-finding study. After acclimatization, the rats began operant training sessions where a single lever-press on either lever (FR1 schedule of reinforcement) resulted in the delivery of one 45 mg food pellet (dustless precision pellets; Bilaney Consultants Ltd, UK). Training sessions lasted for 1 hr or until 50 food pellets were delivered, whichever occurred first. When rats had learnt to press levers on a FR1 schedule, the response requirement was increased to FR2 and the left lever was designated as the active lever. Thereafter, only responses on the left lever resulted in the delivery of food. The response requirement was further increased to a final FR3 schedule.

A dose-finding experiment was performed to identify behaviorally active doses of CBD and diazepam to be used in the self-administration experiment in rats responding under a FR3 schedule. Their effects on general behavior and responding for food (rate of operant

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responding, time to first response on the active lever, total number of responses on the active lever, and reinforcers earned) were determined. Rats received a single i.v. injection of CBD (0.1, 0.5, 2.5 mg/kg; n = 4/group), diazepam (0.03, 0.1, 0.3 mg/kg; n = 4/group) or their vehicles (1.0 ml/kg; n = 5 / Solutol HS15 group; n = 8 / Tween 20 group) immediately prior to test sessions. Based results of the dose-finding experiment, i.v. doses of 0.02, 0.1 and 0.5 mg/kg/infusion CBD were selected for the self-administration experiment based on the lowest dose (0.5 mg/kg) to produce a mild-moderate effect on lever pressing and general behavior, divided as the animals might take multiple infusions per session, to give a starting dose of 0.02 mg/kg/infusion. The subsequent 2 doses of CBD were each increased by 5-fold so that it was tested over a 25-fold dose-range. The starting dose of diazepam was obtained by dividing the lowest dose that produced effects on general behavior (0.03 mg/kg), by 30-fold to 0.001 mg/kg/infusion to provide a starting safety margin given the potent sedative effects of this drug. Diazepam was tested across a 10-fold range of doses in the self-administration experiment. After completion of this experiment, the animals were included in the self-administration experiment.

For statistical analyses, the number of responses on the active lever per minute along with time to first response on the active lever were square root transformed and analyzed by one-way analysis of covariance of square root transformed data with the square root of the baseline value as the covariate. When diazepam was given before sessions, time to the first response on the active lever was analyzed by robust regression of square root transformed data with the square root of the baseline value as the covariate. Each dose of CBD or diazepam was compared to its vehicle group by separate Williams' tests for each compound. Baseline data were the results from the final food training sessions before testing. For total number of responses and reinforcers, comparisons to vehicle were conducted by exact

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Wilcoxon rank sum tests. In the analysis of time to first response on the active lever in rats that received diazepam, one rat had an extremely high value, so robust regression was used to reduce the influence of this value.

Self-administration. After recovery from surgery for at least 6 days, rats were trained to self-administer i.v. heroin in daily 2 hr sessions, which were conducted 5 to 6 days per week. During initial training sessions, 3 responses on the active lever delivered a single i.v. heroin infusion (0.05 mg/kg/infusion). Once robust and consistent responding was achieved under the FR3 schedule, the dose was decreased to 0.015 mg/kg/infusion. Sessions began with a single, non-contingent, i.v. infusion of the solution that was available during the session. Thereafter, when the house light was illuminated, 3 responses on the left lever resulted in the delivery of an i.v. infusion of heroin and initiated a 30-sec time out during which the chamber was dark and responding had no programmed consequence. Rats could respond to receive a maximum of 20 infusions during each session.

Heroin was available for self-administration for at least 14 once-daily sessions and until the criterion for positive reinforcement was achieved (i.e., 3 consecutive sessions with a mean number of heroin infusions of ≥12). Then, saline was available for a minimum of 4 sessions and until responding reached the non-reinforcement criterion (extinction of responding, defined as 3 consecutive sessions with a mean number of infusions of ≤6). Thereafter, CBD (0.02, 0.1 and 0.5 mg/kg/infusion) and the reference comparator diazepam (0.001, 0.003, 0.0045 and 0.01 mg/kg/infusion) were assessed in two separate groups (n=14 for CBD; n=12 for diazepam). Doses of test substances were evaluated from low to high as far as possible. Rats could self-administer up to 2 doses of CBD or up to 3 doses of diazepam until stable responding was achieved, which occurred when the number of infusions/session did not vary by more than ± 20% of the mean of the 3 previous sessions with no obvious increasing or

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decreasing trend in self-administration, or when rats took ≥12 infusions in 3 consecutive sessions, or when rats took ≤6 infusions in 3 consecutive sessions. The maximum number of test sessions for CBD or diazepam was 10. If any of the doses of CBD or diazepam were reinforcing in individual rats, they were given 3-4 saline sessions after their last drug test session to avoid conditioned responding in the animals. After testing all doses of CBD or diazepam, saline was substituted until responding was extinguished, followed by heroin (0.015 mg/kg/infusion).

Statistical methods assumed data were normally distributed with equal variance in the groups. An angular transformation was found to be appropriate. Analysis of the number of infusions was by mixed linear model of angular transformed data with treatment as a fixed factor and animal as a random factor. CBD and diazepam were compared to saline and heroin by separate Dunnett's tests. Heroin was compared to saline by the multiple t test. For this analysis, the means of the number of infusions before and after test substance administration were used for saline and heroin. A separate analysis was employed using the same model, but with separate groups for saline and heroin before and after treatment using the multiple t test to compare responding before and after assessment of the test compound.

Physical dependence and withdrawal studies in rats

Subjects. Juvenile male Sprague Dawley rats (Janvier Labs, Le Genest-Saint-Isle, France) arrived in the laboratory when they were 5 weeks old and weighed 184-240 g whereas adult male rats arrived when they were 10 weeks old and weighed 346-432 g. Juvenile female rats arrived when they were 6 weeks old and weighed 171-224 g whereas adult female rats arrived when they were 11 weeks old and weighed 240-271 g. Rats were housed in groups of 2 animals per cage in macrolon cages (42.5 x 26.6 x 18.5 cm) on wood litter (SERLAB, 60160 Montataire, France) on a 12 h/12 h light/dark cycle at a temperature of 21 ± 3°C and a relative

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humidity ranging from 30-70%. Upon arrival, rats were acclimated for at least 5 days before the study began and allowed free access to water and food (code 113 - SAFE, 89290 Augy, France). Rats were tested in the light part of the light/dark cycle.

Withdrawal study. The method employed for the assessment of withdrawal upon cessation of CBD administration followed that described by Goudie and Leathley (1991). Rats (2 per cage; n=12 rats/group) received twice daily administration (at about 10:00 and 16:00) of CBD-OS (20 or 100 mg/kg p.o.), morphine (64 mg/kg), diazepam (40 mg/kg), or vehicle (placebo oral formulation) for 19 days (study day 1 – study day 19) with the last administration on study day 20 at about 10:00. Beginning on study day 21 and continuing for 8 days, rats received once daily administration of distilled water (p.o.). Control rats received the same number of administrations of vehicle. All drugs were administered via oral gavage.

Starting with the last 3 treatment days (study days 18, 19, and 20) and through the first 8 days following discontinuation of treatment (study days 21-28), food consumption, body weight, and rectal temperature as well as behavioral and physiological manifestations of withdrawal (i.e. jumping, sniffing, wet dog shakes, writhing, ptosis, tremor, genital licks, scratching, hyperactivity, grooming, Straub tail, tiptoe gait, teeth-chattering, dyspnea, diarrhea, and burying) were measured by direct observation immediately prior to the morning drug administration. Rats were first scored for behavioral changes during a 1-minute observation period with 2 animals observed simultaneously after which body weight and rectal temperature were measured. They then received the appropriate treatment and were returned to their home cages. Food remaining in hoppers was weighed immediately after all animals were returned to their cages (mornings only), and their food consumption over the previous 24 hours was calculated. Experimenters were blind to the treatment. Body weight (g), food consumption (g), body temperature (°C), and number of withdrawal signs were averaged across the last 3 days

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of treatment for individual rats to establish a treatment baseline; the same measures were averaged across the first 3 days following discontinuation of treatment. Effects of discontinuation of treatment were quantified using change scores, calculated by subtracting values obtained at the end of treatment from values obtained following discontinuation of treatment. Baseline differences between treatments for each age/sex group were analyzed using raw values and one-way analysis of variance. Effects of discontinuation of treatment were analyzed two ways using change scores. First, a one sample t-test was conducted to determine whether change scores for each group differed from zero, that is, whether data obtained after discontinuation were significantly different from data obtained at the end of treatment. Second, one-way analysis of variance was used to evaluate between-group differences in change scores, and post-hoc comparisons were made using Dunnett's test.

Pharmacokinetic samples and bioanalytical methods

NHP i.v. CBD

Four adult rhesus NHPs (three male and one female) with chronic indwelling i.v. catheters were housed and handled under conditions similar to those described above for NHPs participating in self-administration studies; all had experience with various drugs in previous studies. CBD (0.32, 1.0, and 3.2 mg/kg) was administered i.v. through the access port, followed immediately by a 3-ml of saline flush. Blood (1.0-1.5 ml) was collected in EDTA-containing tubes before and 5, 15, 30, 60, 120, and 240 min after CBD administration by acute puncture of the saphenous vein and was then centrifuged at 0°C and 3000 g for 10 minutes before plasma was collected and stored at -80°C until analysis. Plasma concentrations of CBD and two metabolites, 7-OH-CBD and 7-COOH-CBD, as well as of THC and two metabolites, 11-OH-THC, and 9-COOH-THC, were quantified by HPLC with tandem mass spectroscopic detection (Shimadzu SCL controller, two LC-20AD pumps with a DGU20A degassing unit and

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mixing chamber, SIL-20ACHT autosampler, and an AB Sciex API 4000 Q-trap mass spectrometer with turbo ion spray). The analytical column was an Altima C18 column (Grace & Co, Columbia, Maryland, USA). All solvents and reagents were HPLC grade and purchased from either Fischer Scientific, (Hampton, New Hampshire, USA) or Sigma-Aldrich (St. Louis, Missouri, USA) CBD and deuterated CBD was purchased from Cayman Chemical (Ann Arbor, Michigan, USA).

Rat i.v. CBD

Male, Sprague-Dawley rats (250-275g) were purchased from Charles River (Kent, England, UK) and housed as described above for the i.v. drug self-administration study except that 3 to 4 were housed in each cage and they had free access to rodent chow. On study day, CBD was administered and blood samples (~300 µl) collected via a tail vein in restrained, conscious rats; to avoid cross-contamination, blood samples were collected from a different tail vein. Blood samples were taken at 2.5, 10, 15, and 30 minutes. Doses of CBD (0.081, 0.808 and 2.67 mg/kg; n=3 for each dose) were calculated from the mean number of injections taken over the final 3 test sessions (plus the 1 non-contingent injection) for each of the 3 doses of CBD used in the self-administration study. Blood samples were centrifuged (5000 rpm for 5 min at 4°C) and plasma was stored at -80ºC until analyzed for CBD concentrations. Methods using UPLC-MS/MS were validated with respect to linearity, precision, accuracy, and stability for the analysis of CBD and its metabolites 6-OH-CBD, 7-OH-CBD, 7-COOH-CBD. Negative controls and calibration standards were processed then injected into an Acquity Binary Solvent Manager UPLC-MS/MS at a flow rate of 0.6 ml/minute with a run time of 6.8 minutes at 65^{o} C (mobile phases comprising 0.1% ammonia in methanol and 5 mM aqueous ammonium formate ,pH 9) and connected to an Applied Biosystems API5000 mass spectrometer with a

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heated nebulizer at 500° C (amps) and the following parameters: gas pressure, 40 psi; curtain gas pressure, 35 psi; collision gas pressure, 7 psi; probe position, 5 mm / 5 mm.

Rat p.o. CBD

Female, Lister Hooded rats, purchased from Charles River (Kent, England, UK) and housed as described above for drug discrimination studies, were used for plasma analysis following p.o. administration of CBD. Blood samples (~250 µl) were drawn from a tail vein immediately before as well as 60, 120, and 150 min after administration of 20, 75, or 150 mg/kg CBD (n = 3 rats per dose). Blood samples were centrifuged (5000 rpm for 5 min at 4°C) and plasma was stored at -80ºC until analyzed for CBD concentrations.

RESULTS

Dose-finding and self-administration studies in NHPs

NHPs responded for food with average response rates ranging from 1.0 to 1.3 responses per second across cycles following vehicle (i.v.) treatment before and after tests with CBD. Pretreatment with 5.6 mg/kg CBD (i.v.) decreased average response rate to 0.6 responses per second during the first cycle of the session (i.e., 15 min after pretreatment) but rates were not altered at later time points following this dose or at any time point after treatment with 1.0 or 3.2 mg/kg CBD (Table 1).

When midazolam (0.01 or 0.032 mg/kg/infusion, depending on the subject) was available for self-administration, NHPs obtained on average 12.9, 13.2, and 11.5 infusions per session at the beginning, middle, and end of the experiment, respectively (Figure 1). Vehicle maintained very little responding, with NHPs obtaining on average 1.1, 0.3, and 0.6 infusions per session at the beginning, middle, and end of the experiment, respectively. CBD also maintained very little responding, with NHPs obtaining on average 0.5, 0.6, 0.5, and 0.2

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infusions per session for unit doses of 0.1, 0.32, 1.0, and 3.2 mg/kg/infusion, respectively. There was a significant effect of condition ($F[1.46, 5.84] = 36.71$, $p=0.0007$, G-G epsilon = 0.18), with the number of infusions obtained during all three tests of midazolam and no tests of CBD being significantly different from the first vehicle test ($p<0.05$).

Drug discrimination studies in rats

None of the doses of CBD (20, 75, and 150 mg/kg p.o.) significantly altered the rate of operant responding (Table 2) and no general behavioral effects of the compound were observed. CBD (20, 75, and 150 mg/kg p.o.; n=6) did not generalize to the midazolam discriminative stimulus (0.5 mg/kg, i.p.). All 3 doses of CBD administered p.o. yielded mean values meeting the criterion for generalization to saline (Figure 2, top panel). At 20 mg/kg CBD, 5 of 6 rats generalized to saline and 1 of 6 partially generalized to midazolam (30.8%). At the 75 mg/kg and 150 mg/kg CBD, the values for all 6/6 rats in each group showed generalization to saline.

Alprazolam administered p.o. dose-dependently increased midazolam-lever responding (Figure 2, bottom panel). Rats responded 11.1 ± 11.9% on the midazolam lever with 0.125 mg/kg alprazolam, meeting the criterion for generalization to saline; 5 of 6 rats generalized to saline (<25% midazolam-lever responding) and 1 of 6 rats generalized partially to midazolam (31.9%). A dose of 0.25 mg/kg alprazolam produced partial generalization to midazolam, with 3/6 rats generalizing to saline, 1 of 6 rats generalizing partially to midazolam (33.7%), and 2 of 6 rats generalizing fully to midazolam (83.7% and 97.2%). A dose of 0.50 mg/kg alprazolam produced 86.3 ± 22.8% midazolam-lever responding, indicating full generalization. At this dose, 1 of 6 rats generalized partially (40.2%) and 5 of 6 rats fully (97.5, 91.6, 94.3, 94.9 and 99.5%) to midazolam. The largest alprazolam dose (1.0 mg/kg) also generalized fully (89.0 ± 16.5%), with 1 of 6 rats showing partial generalization to midazolam (56.5%) and 5 of 6 rats

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showing full generalization (87.2, 95.6, 96.9, 98.9 and 99.0%). Up to the largest dose tested, alprazolam did not alter the number of responses emitted during the test session (0.5 mg/kg midazolam (i.p.) = 360.1 lever presses; 1.0 mg/kg alprazolam (p.o.) = 377.8 lever presses).

Drug self-administration studies in rats

CBD (0.1, 0.5, 2.5 mg/kg) administered i.v. did not significantly alter rate of active lever pressing (Table 3), time to make the first active lever press (Table 4), or total number of active lever presses or food reinforcers obtained (data not shown) when compared with the vehicle group. There were general, non-significant trends for CBD to increase rate of active lever pressing for food by 162.4% at 0.5 mg/kg (Table 3) and decrease the time to the first active lever press to 47.5% (Table 4) compared with vehicle. At 2.5 mg/kg, there was a general trend to increase the time to first lever press by 144.5% (not significant; Table 4). CBD produced mild effects on general behavior at all doses, which lasted up to 60 min, including altered locomotor activity, mild ataxia, sniffing, rearing and subdued behavior. Similarly, there were no significant effects of diazepam (0.03, 0.1, 0.3 mg/kg) administered i.v. on rate of active lever pressing (Table 3) and total number of active lever presses and food reinforcers obtained (data not shown) compared with vehicle. There was a dose-dependent effect of diazepam to increase the time to make the first active lever press by 284.0% ($p=0.002$; Table 4) at the highest dose tested compared with the vehicle. Diazepam produced other behavioral effects at all doses tested, including mild to moderate subdued behavior and ataxia, flat posture, slow movements and sniffing lasting up to 30 min.

Heroin (0.015 mg/kg/infusion) maintained robust self-administration (Figure 3, Table 5), which was significantly greater than the saline group mean; statistically adjusted values of 17.6 ± 0.5 versus 3.7 ± 0.2, respectively ($p<0.001$; n=39/group for pre- and post-testing values combined). In the group of rats subsequently used to evaluate CBD (n=14), heroin maintained

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high levels of heroin self-administration before and after testing CBD at 18.5 ± 0.6 infusions/session versus 17.1 ± 1.0 infusions/session, respectively (Table 5). In the diazepam group (n=12), heroin maintained 18.6 ± 0.9 infusions/session pre-testing and 17.2 ± 1.1 infusions/session post-testing (Table 4). Hence, the self-administration of heroin during acquisition and reinstatement was not different for each of the 2 groups of rats (the remaining 13 rats of the total group of 39 were used to assess a separate test compound that is not the subject of this article).

Only the 0.1 mg/kg/infusion CBD dose (n = 8) maintained self-administration at 6.9 ± 1.8 infusions/session ($p<0.05$ versus saline responding) (Figure 3). For the other 2 CBD doses tested, the statistically adjusted mean number of infusions/session of 0.02 mg/kg/infusion CBD (n = 8) ~~obtained~~ was 2.8 ± 0.7 ~~infusions/session~~ ~~(n = 8~~) and for 0.5 mg/kg/infusion CBD (n = 8) was 4.0 ± 1.1 ~~infusions/session~~ ~~(n = 8)~~ (Figure 3 ~~2~~). When the responses of the individual rats were analyzed, the number of animals per group that self-administered CBD at >6 infusions/session were 1 of 8 for 0.02 mg/kg/infusion CBD, 3 of 8 for 0.1 mg/kg/infusion CBD, and 2 of 8 for 0.5 mg/kg/infusion CBD. The number of infusions per session obtained for all 3 doses of CBD was significantly lower ($p<0.001$ all doses) than those taken for heroin (17.6 ± 0.5 infusions/session, n = 39). CBD met the criterion for serving as a weak positive reinforcer at 1 dose out of the 3 doses tested.

The 0.003 mg/kg/infusion dose of diazepam (n = 8) maintained statistically adjusted responding of 7.0 ± 2.1 infusions/session, which was significantly greater ($p<0.05$) than responding for saline (Figure 3). None of the other doses of diazepam (0.001, 0.0045 and 0.01 mg/kg/infusion; n = 7, 8, and 7 rats/group, respectively) maintained self-administration at >6 infusions session, or at levels significantly greater than saline (Figure 3). The statistically adjusted mean number of infusions of 0.001 mg/kg/infusion was 3.4 ± 0.6 infusions/session (n

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= 7), 0.0045 mg/kg/infusion was 4.8 ± 1.5 infusions/session (n = 8), and 0.01 mg/kg/infusion was 4.3 ± 1.5 infusions/session (n = 7). When the responses of the individual rats were analyzed, the number of animals that self-administered diazepam > 6 infusions/session were 0 of 7 for 0.001 mg/kg/infusion, 4 of 8 for 0.003 mg/kg/infusion, 2 of 8 for 0.0045 mg/kg/infusion, and 3 of 7 for 0.01 mg/kg/infusion. The number of infusions per session obtained for all 4 doses of diazepam was significantly lower ($p<0.001$ all doses) than those obtained for heroin (17.6 ± 0.5 infusions/session, n = 39; Figure 3). Therefore, diazepam was a weak reinforcer at 1 of the 4 doses tested.

Physical dependence and withdrawal studies in rats

Adult and juvenile male rats as well as adult female rats treated twice daily with 64 mg/kg morphine gained less weight compared with age- and sex-matched rats treated with vehicle; juvenile female rats treated with 100 mg/kg CBD gained more weight compared with female rats treated with vehicle (Table 6; see Supplemental Table 1 for accompanying statistics). All rats treated with 40 mg/kg diazepam consumed more food compared with vehicle treated rats. Morphine treatment increased food consumption in adult male, adult female, and juvenile female rats, whereas treatment with 100 mg/kg CBD increased food consumption in juvenile male and female rats. Adult male, juvenile male, and adult female rats treated with diazepam had higher body temperatures compared with vehicle-treated rats, as did adult male rats treated with 100 mg/kg CBD. Morphine treatment decreased body temperature in adult and juvenile females. No groups differed from vehicle-treated groups in the number of withdrawal signs observed at the end of their respective daily treatment regimen.

Following discontinuation of treatment, changes in body weight were significantly greater in morphine-treated rats compared with vehicle-treated rats (top row, Figure 4 see

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Supplemental Table 2 for accompanying statistics); all groups of vehicle-treated rats gained weight (change scores significantly greater than zero, $p<.05$), whereas all groups of morphine-treated rats lost weight (change scores significantly less than zero). Adult females treated with 100 mg/kg CBD or 40 mg/kg diazepam also lost weight following discontinuation of treatment; however, the magnitude of weight loss appeared smaller than in morphine-treated rats. Change in food intake in all groups of morphine- and diazepam-treated rats differed from vehicle-treated rats (middle row, Figure 4). Food intake increased in all groups of vehicle-treated rats and decreased in all groups of morphine-treated rats; food intake also decreased in all groups of diazepam-treated rats except adult males for which there was no change. Change in food intake in juvenile females treated with 100 mg/kg CBD also differed from vehicle-treated controls because there was no difference in intake following discontinuation of treatment. Changes in body temperature were less consistent across groups (bottom row, Figure 4); adult males as well as adult and juvenile females treated with diazepam differed from vehicle-treated controls, as did adult females treated with 100 mg/kg CBD. All groups of morphine-treated rats, except juvenile females, displayed significantly increased withdrawal signs (change scores greater than zero) that were different from change scores in vehicle-treated rats (Figure 5; see Supplemental Table 2 for accompanying statistics). Adult females also displayed significantly increased withdrawal signs following discontinuation of treatment with 20 mg/kg CBD but scores in this group did not differ from the vehicle-treated group.

Pharmacokinetic studies

NHP i.v. CBD

In NHPs, plasma levels of CBD peaked within 5 minutes of i.v. administration, with C_{max} averaging 478, 1730, and 5530 ng/ml after injection of 0.32, 1.0, and 3.2 mg/kg CBD, respectively (Supplemental Figure 1); the plasma half-life of CBD following administration of

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3.2 mg/kg was estimated to be 44.4 min. Plasma concentrations of metabolites 7-OH-CBD and 7-COOH-CBD peaked within 15 minutes of CBD injection and were generally related to dose of CBD (Table 4), with longer half-lives (103.2 and 83.9, respectively) compared with CBD. Plasma concentrations for THC and its metabolites, 9-COOH-THC and 11-OH-THC, were below the limits of detection.

Rat i.v. CBD

In rats, plasma C_{max} concentrations of CBD following i.v. administration increased in a dose-dependent manner (Supplemental Figure 2) with the T_{max} being 2.5 min for each CBD dose. Plasma C_{max} concentrations of CBD were 50.8, 467.0 and 1513.0 ng/ml (n = 3/dose) after injection of 0.081, 0.808 and 2.67 mg/kg CBD, respectively (the number of infusions self-administered each session, plus the non-contingent injection at the start of the session). Clinical plasma C_{max} CBD values were 356 ng/ml for a 750 mg p.o. dose and 618 ng/ml for a 1500 mg p.o. dose (data on file GW Pharmaceuticals). Therefore, the C_{max} after i.v. injection of the equivalent mean accumulated drug intake for the doses of CBD ranged between fractions of 0.08x to 2.5x of the highest clinical C_{max} value in accordance with the FDA Guidance (2017). Plasma levels of metabolites 7-OH-CBD, 7-COOH-CBD, and 6-OH-CBD increased dose-dependently as a function of CBD dose, reaching peak concentrations within 10 min of CBD injection.

The limit of detection in the assay for THC was 0.25 ng/ml, and for 11-COOH-THC and 11-OH-THC the limit of detection was 0.5 ng/ml. The levels of THC at 2.5 min post dosing (Tmax) were below the limit of quantitation (BLQ) for 0.081 mg/kg CBD (n=3), 0.384 ng/ml for 0.808 mg/kg CBD (n=1; BLQ n=2), and 0.812 ng/ml for 2.67 mg/kg CBD (n=3). The THC levels were BLQ for all animals by 10 min post-dosing. The plasma levels of 11-COOH-THC and 11-

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OH-THC were BLQ for all animals (n=3/dose). None of the analytes assayed were reported as above the limit of quantitation.

Rat p.o. CBD

In rats, plasma concentrations of CBD increased in a dose-dependent manner following p.o. administration resulting in CBD concentrations of 578, 1262, and 2907 ng/ml 120 min (Tmax) following administration of 20, 75, and 150 mg/kg, respectively (Supplemental Figure 3). These values equated to 0.94x, 2.04x and 3.94x multiples of the clinical Cmax. Plasma levels of the metabolites 7-OH-CBD, 7-COOH-CBD, and 6-OH-CBD also increased in a dose-dependent manner with levels obtained 120-150 min after injection of CBD being, generally, higher than those obtained 60 min after injection.

The limit of detection was 0.25 ng/ml for THC and 0.5 ng/ml for 11-COOH-THC and 11-OH-THC. The levels of THC at 2 hr post CBD dosing (Tmax) were 0.631 ng/ml for 20 mg/kg CBD, 0.972 ng/ml for 75 mg/kg CBD, and 1.66 ng/ml for the 150 mg/kg CBD (n=3/dose). The plasma levels of 11-COOH-THC and 11-OH-THC were BLQ for all animals (n=3/dose), except for the 3 hr time-point of the 150 mg/kg CBD group where the value was 0.573 ng/ml. None of the analytes were above the limit of quantitation.

DISCUSSION

Cannabis and cannabinoid drugs are widely used to treat disease or alleviate symptoms, but their efficacy for specific indications is not clear. Underpinned by the diverse pharmacology of the constituent compounds, cannabinoids present a significant opportunity to address unmet clinical need in conditions refractory to conventional pharmaceuticals. CBD represents the most plausible example of such a compound in that it possesses negligible pharmacological activity at the CB1 receptors, the activation of which is primarily responsible

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for the psychoactive effects of THC. Instead, CBD possesses a far-reaching pharmacological profile, engaging many druggable molecular targets, albeit with low affinity and/or potency. It is this low potency at a wide range of targets that likely confers a multitude of potential efficacies in multiple diseases and a favorable safety profile.

In the present study, the abuse potential of CBD was assessed in robust preclinical models of reward, affect, and physical dependence/withdrawal. These studies represent a major component of the panel of studies designed to predict the potential of a compound, which has access to the CNS, to be abused in humans. Such studies are complimented by *in vitro* affinity and functional studies in which the ability of increasing concentrations of compound to displace specific binding at targets associated with abuse has been assessed.

The suggestion that CBD appears to possess benzodiazepine-like properties was borne from the observation that somnolence was observed in patients receiving therapeutic doses of CBD (20 mg/kg p.o.) although CBD did not bind to orthosteric or benzodiazepine sites of the $GABA_A$ receptor *in vitro*. Self-administration and drug discrimination studies in midazolam-trained animals revealed that CBD has neither reinforcing properties in NHPs that self-administer midazolam nor discriminative stimulus effects in rats trained to discriminate midazolam. It is of note that the somnolence observed was in refractory epilepsy patients whose concomitant medication(s) included a benzodiazepine, most commonly clobazam. Like CBD, clobazam is a cytochrome CYP3A4 substrate. The effect could be an increase in circulating clobazam levels resulting in more somnolence (Huestis et al. 2019). Other evidence suggestive of the non-benzodiazepine effect comes from a separate study in schizophrenic patients who were not taking benzodiazepines, where no such treatment-related somnolence was observed (McGuire et al., 2017).

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It is important to note that while CBD examined here is purified to ≥ 98% (w/w), the API contains up to 0.1% THC (w/w). Therefore, it was important to determine the circulating THC concentration following doses producing therapeutic CBD exposures. While the exposures measured in each study were equal to or greater than plasma CBD concentrations measured in patients (Devinsky et al., 2018) the concentrations of THC measured either were below the level of quantitation or significantly lower than the known affinity of THC at CB1 receptors. It is an interesting proposal that CBD, instead of representing a risk to patients in terms of abuse potential, may possess properties that could be of utility for treating substance use disorders, specifically the symptoms of dysphoria associated with the pre-relapse phase of drug abuse.

Taken together, the lack of reliable self-administration, the failure to increase drug-lever responding in rats trained to discriminate midazolam, and the absence of withdrawal signs upon discontinuation of chronic treatment indicate that CBD has very low abuse potential and is unlikely to produce physical dependence. These data are consistent with abuse potential studies in humans indicating that CBD has negligible abuse-related effects. For example, in healthy, frequent marijuana users, oral synthetic CBD (up to 800 mg) did not increase subjective ratings of drug effect that would be indicative of abuse potential, including street value estimates and ratings of high, feeling good drug effect, desire to take again, sedated, and mellow (Babalonis et al. 2016; see also a recent study by Arout et al. 2021). Likewise, in healthy recreational polydrug users, a therapeutic dose of the oral Epidiolex formulation of CBD (750 mg) did not produce any signs of abuse potential (Schoedel et al. 2018).

Moreover, there is a growing body of evidence supporting the notion that CBD could be an effective therapy for treating substance use disorders, including relapse prevention, and that CBD itself has no potential for abuse. For example, in individuals with heroin use disorder, acute CBD administration significantly reduced craving and anxiety induced by the

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presentation of drug-associated cues and attenuated drug cue–induced changes in heart rate and salivary cortisol levels, suggesting it might be useful for preventing relapse in opioid use disorder (Hurd et al., 2019). CBD might have potential for treating cannabis use disorder (Freeman et al. 2020), cigarette smoking (Morgan et al. 2013), as well as alcohol use disorder (see review by Nona et al. 2019).

In summary, in a robust, comprehensive assessment of the abuse potential of highly purified CBD API or CBD-OS, the clinical formulation of Epidiolex, no rewarding properties, withdrawal potential, or similarity to a benzodiazepine ~~or THC were~~ was observed. Therefore, taking these data together with those generated in *in vitro* pharmacology and human abuse potential studies, the abuse potential of Epidiolex in humans is likely to be negligible.

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: RAG, DJH, DRM, LRG, MAJ, SM, CPF

Conducted experiments: DRM, LRG, MAJ, SM

Performed data analyses: DJH, DRM, LRG, MAJ, SM

Wrote or contributed to the writing of the manuscript: RAG, DJH, DRM, LRG, MAY, SM, CPF

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RAG was an employee of GW Pharmaceuticals when these studies were conducted. These studies were supported by GW Pharmaceuticals

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FINANCIAL DISCLOSURE

No author has an actual or perceived conflict of interest with the contents of this article.

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FIGURE CAPTIONS

Figure 1. Lack of positive-reinforcing effects of CBD in 3 female and 2 male rhesus monkeys that self-administered midazolam. The number of infusions obtained per session for each monkey, averaged over the last 3 sessions of each condition, is plotted for each condition. The maintenance dose of midazolam was 0.032 mg/kg/infusion in two monkeys and 0.01 mg/kg/infusion in three monkeys; midazolam and vehicle were available at the beginning, middle, and end of the experiment. Horizontal lines indicate the group mean. Asterisks indicate conditions that are significantly different from the first vehicle test according to a Dunnett's post hoc test ($p<0.05$).

Figure 2. Substitution of alprazolam but not CBD in female Lister Hooded rats trained to discriminate 0.5 mg/kg midazolam (n=6). Percentage of responses emitted on the midazolam-associated lever is plotted across test conditions. Points above "Saline" and "Midazolam" indicate effects of saline and the training dose of midazolam (0.5 mg/kg), respectively, administered i.p. under test conditions. Triangles (top) show data from tests with CBD administered p.o. 120 minutes prior to the session, whereas diamonds (bottom) show data from tests with alprazolam administered p.o. 60 minutes prior to the session. Data points show the mean, and error bars show 1 standard error of the mean.

Figure 3. Reinforcing effect of CBD and diazepam in rats trained to self-administer heroin under a FR-3 schedule. The number of infusions is plotted across test conditions. Points above "Saline" and "Heroin" indicate the number of infusions obtained when saline and 0.015 mg/kg/infusion heroin, respectively, were available for self-administration. Data points show the mean, and error bars show 1 standard error of the mean. CBD (0.02, 0.1 or 0.5 mg/kg/infusion), n = 8/group. Diazepam (0.001, 0.003, 0.0045 or 0.01 mg/kg/infusion), n=7, 8,

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8 or 7/group, respectively. Most animals were tested at more than one dose of CBD and diazepam. Heroin (n=39) and saline (n=39) values are the combined mean of the pre-test substance and post-test substance dose values. Significantly different to saline *p<0.05, ***p<0.001. Significantly different from heroin ###p<0.001.

Figure 4. Change scores for body weight (top row), food consumption (middle row), and body temperature (bottom row) in male and female, juvenile and adult rats following discontinuation of twice daily treatment with vehicle, 20 mg/kg CBD, 100 mg/kg CBD, 40 mg/kg diazepam, or 64 mg/kg morphine. Change scores were calculated by taking the difference between the average of the last 3 days of daily treatment and the average of the first 3 days following discontinuation of treatment. Data points show scores for individual rats and horizontal lines show the group mean. Asterisks indicate groups whose change scores differed from zero according to a one-sample t-test (p<0.05); pound signs indicate groups that differed from vehicle-treated age- and sex-matched controls according to a Dunnett's post-hoc test (p<0.05).

Figure 5. Change scores for withdrawal signs in male and female, juvenile and adult rats following discontinuation of twice daily treatment with vehicle, 20 mg/kg, CBD 100 mg/kg CBD, 40 mg/kg diazepam, or 64 mg/kg morphine. Other details are the same as in Figure 4.

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TABLES

Table 1. Results from the NHP dose finding study. Mean (± 1 SEM) response rates in responses per second are shown for each pretreatment condition and time point as well as session mean (n=2).

	Time (min)				
Pretreatment	15	30	45	60	Session
Vehicle (before)	1.0 (0.02)	1.1 (0.09)	1.1 (0.13)	1.2 (0.02)	1.1 (0.04)
1.0 mg/kg CBD	1.1 (0.09)	1.1 (0.25)	1.3 (0.19)	1.1 (0.02)	1.1 (0.06)
3.2 mg/kg CBD	0.9 (0.57)	1.0 (0.20)	1.2 (0.08)	1.1 (0.08)	1.1 (0.09)
5.6 mg/kg CBD	0.6 (0.11)	1.0 (0.09)	1.2 (0.07)	1.1 (0.19)	1.0 (0.20)
Vehicle (after)	1.3 (0.07)	1.2 (0.02)	1.1 (0.09)	1.2 (0.01)	1.2 (0.06)

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Table 2. Results of the p.o. dose-finding study in rats. Effects of CBD on total number of lever presses for milk.

Treatment	n	Mean	SEM	% of vehicle	p
Vehicle (Placebo Oral Solution, 2 ml/kg)	5	253.8	38.1		
20 mg/kg CBD	5	297.5	33.0	117.3	0.957
75 mg/kg CBD	5	255.6	30.3	100.7	0.957
150 mg/kg CBD	5	255.8	33.8	100.8	0.957
Ten minute test sessions began 120 min after drug administration.					

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Table 3. Results of the i.v. dose-finding study in rats. Effects of CBD and diazepam on response rate (presses/min) for food pellets.

Treatment	n	Mean	SEM	% of vehicle	p
Vehicle (5% Solutol HS15, 1 ml/kg)	5	11.5	1.8		
0.1 mg/kg CBD	4	12.7	1.8	110.4	0.71
0.5 mg/kg CBD	4	18.7	2.4	162.4	0.50
2.5 mg/kg CBD	4	10.6	2.8	92.4	0.50
Vehicle (2% Tween 80, 1 ml/kg)	8	8.9	1.9		
0.03 mg/kg diazepam	4	10.1	1.8	113.7	0.66
0.1 mg/kg diazepam	4	12.1	1.2	136.7	0.55
0.3 mg/kg diazepam	4	10.0	3.2	113.2	0.55

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Table 4. Results of the i.v. dose-finding study in rats. Effects of CBD and diazepam on time (seconds) to first press on the active lever for food pellets.

Treatment	n	Mean	SEM	% of vehicle	p
Vehicle (5% Solutol HS15, 1 ml/kg)	5	29.9	4.0		
0.1 mg/kg CBD	4	29.2	8.0	97.7	1.00
0.5 mg/kg CBD	4	14.2	11.8	47.5	1.00
2.5 mg/kg CBD	4	43.3	14.9	144.5	0.58
Vehicle (2% Tween 80, 1 ml/kg)	8	19.6	3.1		
0.03 mg/kg Diazepam	4	18.3	1.1	93.2	1.00
0.1 mg/kg Diazepam	4	25.5	3.3	130.0	0.57
0.3 mg/kg Diazepam	4	55.7	93.2	284.0	0.002**
**p<0.01					

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Table 5. Comparison of saline and heroin (0.015 mg/kg/infusion) infusions obtained before and after CBD and diazepam testing.

Treatment	n	Mean	SEM	p
CBD				
Saline extinction before CBD test	14	3.4	0.4	
Saline extinction after CBD test	13	3.2	0.4	0.83
Heroin before CBD test	14	18.5	0.6	
Heroin after CBD test	13	17.1	1.0	0.09
Diazepam				
Saline extinction before diazepam test	12	4.2	0.3	
Saline extinction after diazepam test	12	4.0	0.5	0.88
Heroin before diazepam test	12	18.6	0.9	
Heroin after diazepam test	12	17.2	1.1	0.19

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Table 6. Baseline measures of body weight, food consumption, body temperature, and observable signs in rats treated twice daily with vehicle, CBD, diazepam, or morphine. Values indicate the mean (± 1 SEM) of the last 3 days of treatment (days 18, 19, and 20) separated by age, sex, and treatment condition. See Supplemental Table 1 for accompanying statistics.

				Body weight	Food consumption	Temperature	Observable signs
Age	Sex	Daily treatment	n	(g)	(g)	(°C)	(#)
Adult	Male	Vehicle	12	488.5 (8.5)	16.3 (0.4)	36.2 (0.04)	0.22 (0.09)
Adult	Male	20 mg/kg CBD	9	495.9 (6.8)	17.26 (0.2)	36.2 (0.05)	0.07 (0.05)
Adult	Male	100 mg/kg CBD	11	490.4 (11.8)	16.99 (0.3)	36.5 (0.06)*	0.06 (0.04)
Adult	Male	40 mg/kg diazepam	10	487.2 (12.2)	24.86 (2.0)*	36.8 (0.10)*	0.47 (0.10)
Adult	Male	64 mg/kg morphine	11	418.7 (9.6)*	21.98 (1.0)*	36.4 (0.08)	0.24 (0.08)
Juvenile	Male	Vehicle	12	371.2 (5.7)	22.49 (0.5)	36.9 (0.06)	0.39 (0.12)
Juvenile	Male	20 mg/kg CBD	12	373.3 (5.1)	22.74 (0.5)	37.1 (0.08)	0.17 (0.06)
Juvenile	Male	100 mg/kg CBD	12	377.9 (5.5)	24.24 (0.3)*	36.9 (0.05)	0.22 (0.06)
Juvenile	Male	40 mg/kg diazepam	12	375.8 (7.0)	29.47 (0.5)*	37.5 (0.12)*	0.50 (0.10)
Juvenile	Male	64 mg/kg morphine	11	302.1 (5.4)*	22.79 (0.2)	36.9 (0.03)	0.15 (0.09)
Adult	Female	Vehicle	12	285.0 (3.6)	11.82 (0.4)	37.1 (0.13)	0.44 (0.09)
Adult	Female	20 mg/kg CBD	12	291.9 (4.8)	11.73 (0.1)	37.3 (0.14)	0.25 (0.08)
Adult	Female	100 mg/kg CBD	12	297.9 (3.6)	12.92 (0.3)	36.9 (0.07)	0.28 (0.07)
Adult	Female	40 mg/kg diazepam	12	286.3 (3.0)	19.76 (0.5)*	37.7 (0.10)*	0.47 (0.11)
Adult	Female	64 mg/kg morphine	11	267.3 (4.5)*	17.42 (0.3)*	36.5 (0.05)*	0.18 (0.07)
Juvenile	Female	Vehicle	12	259.4 (5.1)	13.69 (0.3)	37.4 (0.15)	0.81 (0.13)
Juvenile	Female	20 mg/kg CBD	12	252.1 (3.2)	13.85 (0.2)	37.0 (0.16)	0.89 (0.11)
Juvenile	Female	100 mg/kg CBD	12	286.3 (6.2)*	17.52 (0.4)*	37.3 (0.14)	0.56 (0.16)
Juvenile	Female	40 mg/kg diazepam	12	259.0 (4.7)	20.03 (0.3)*	37.9 (0.13)	1.06 (0.16)
Juvenile	Female	64 mg/kg morphine	12	244.1 (1.1)	17.91 (0.2)*	36.6 (0.07)*	0.61 (0.17)

Asterisks indicate conditions that are statistically different from vehicle within each age/sex group according to a Dunnett's post-hoc test ($p<.05$)

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Figure 1

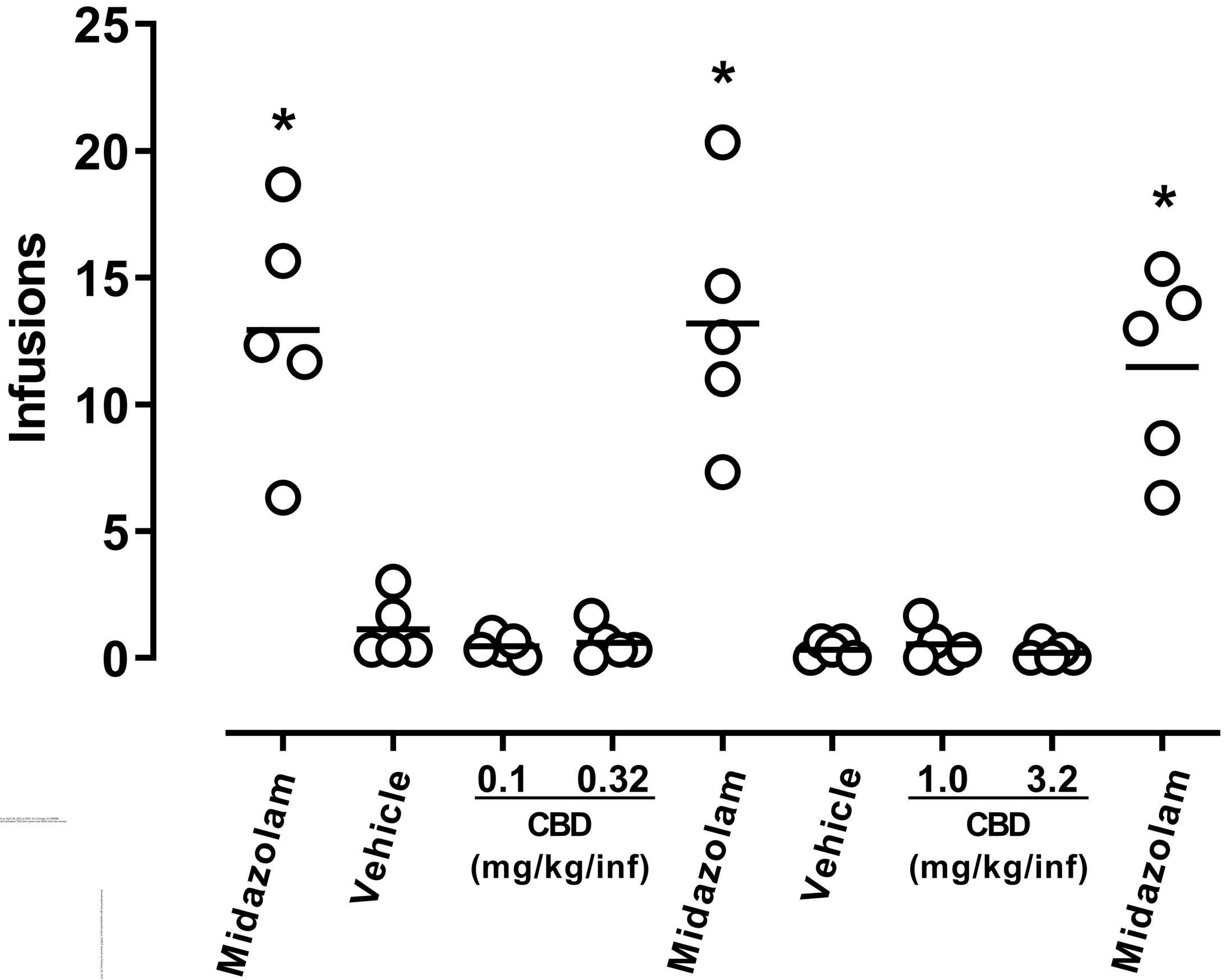

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Figure 2

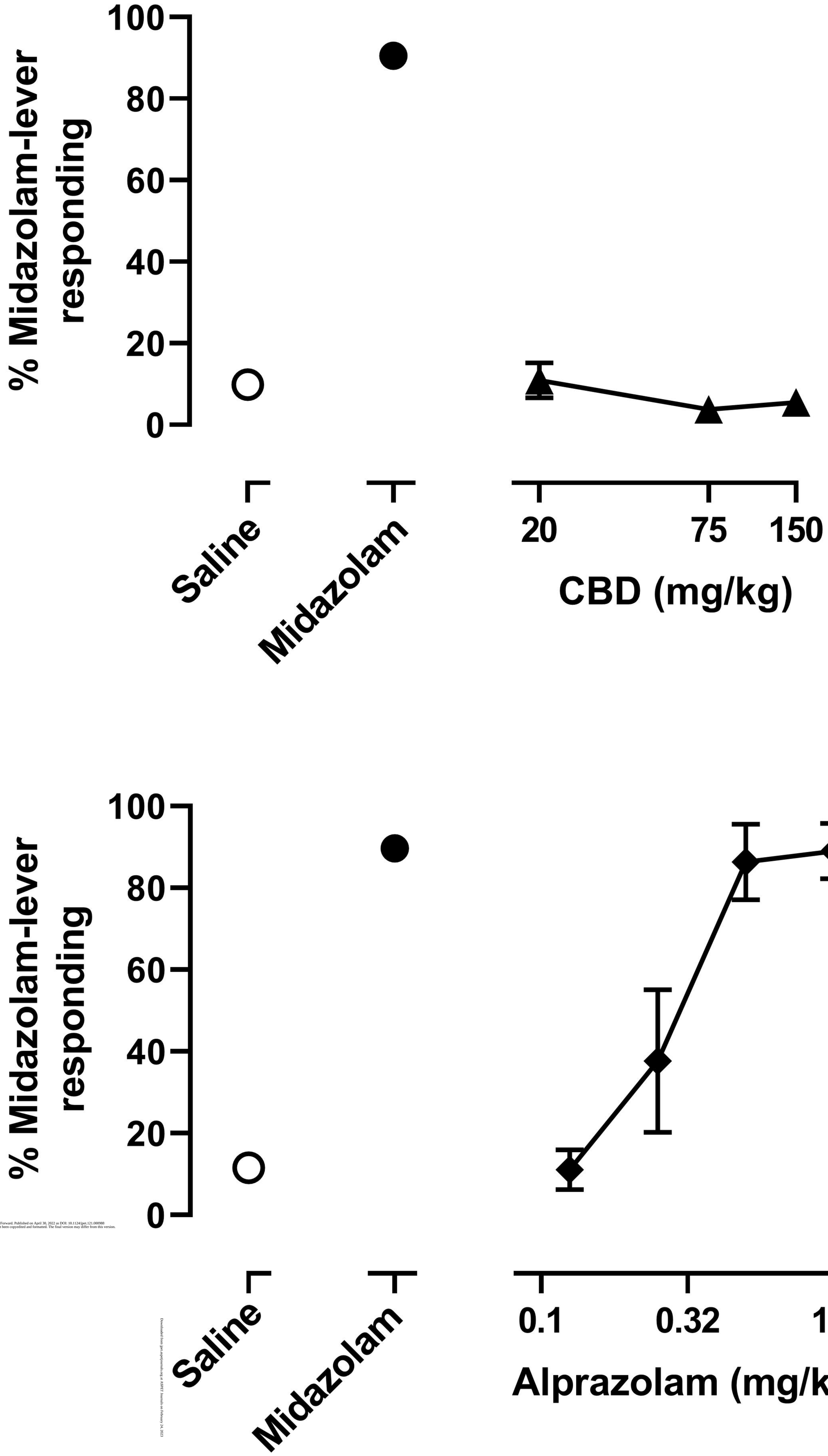

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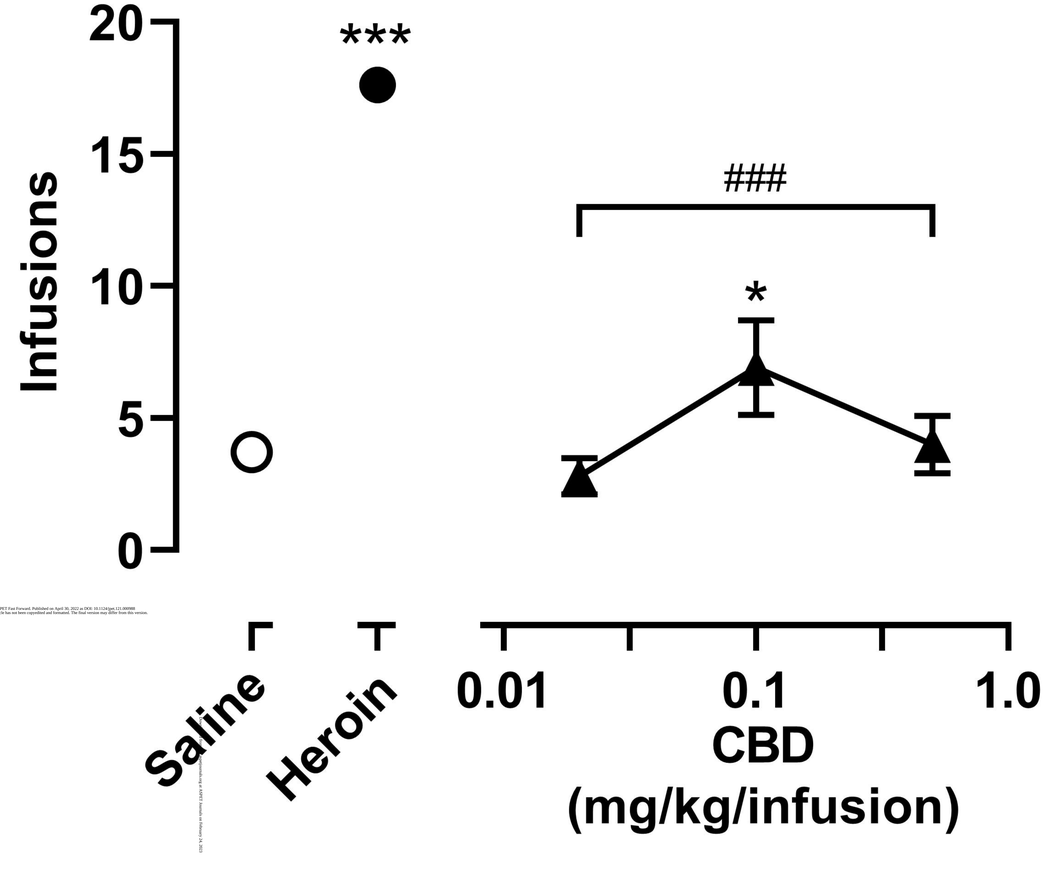
Figure 3



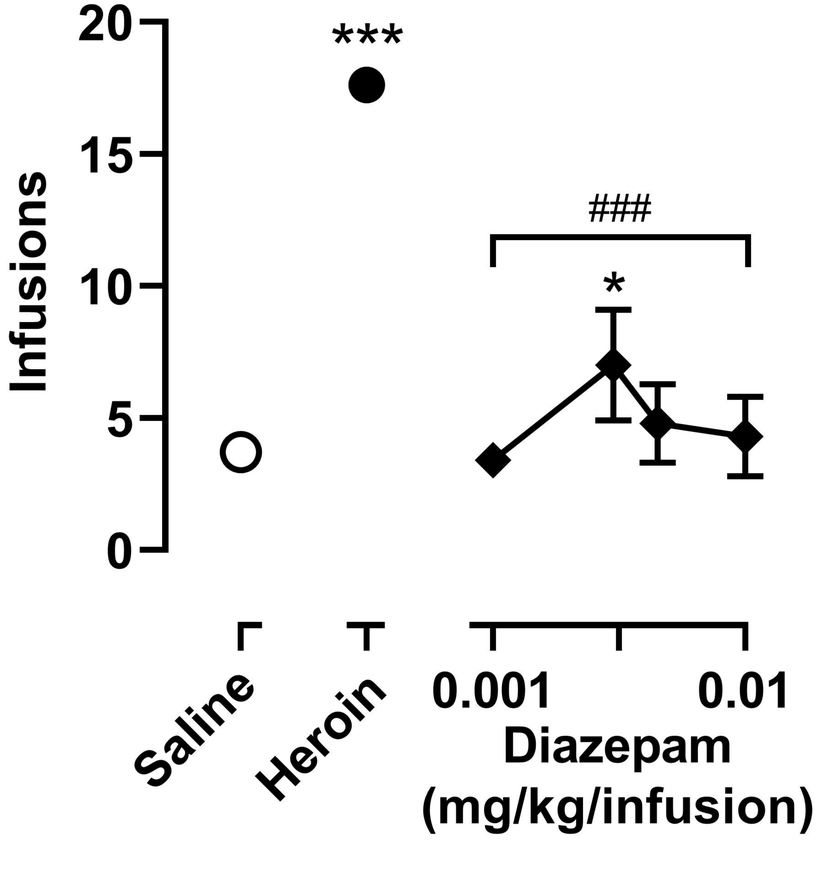


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Figure 4

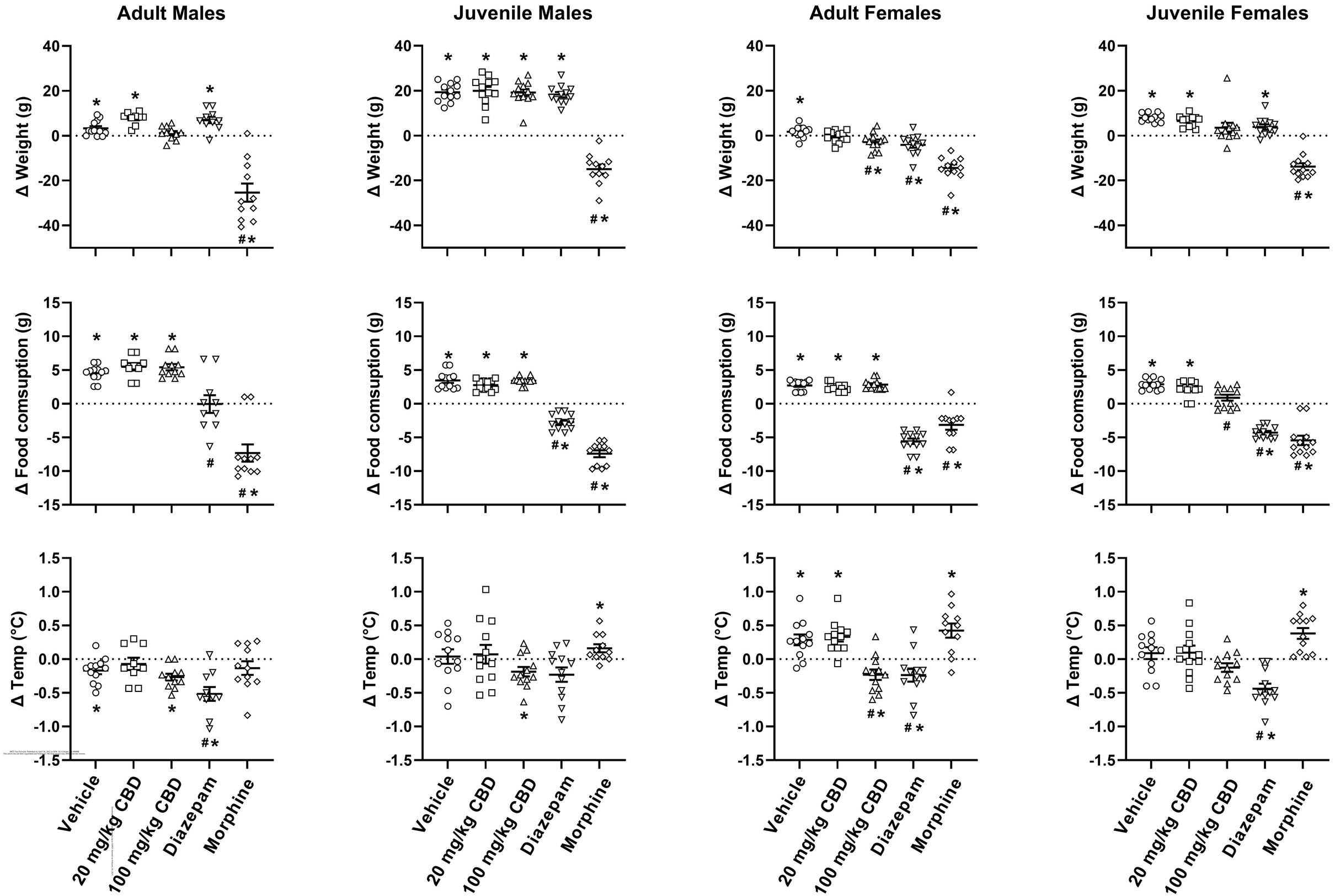
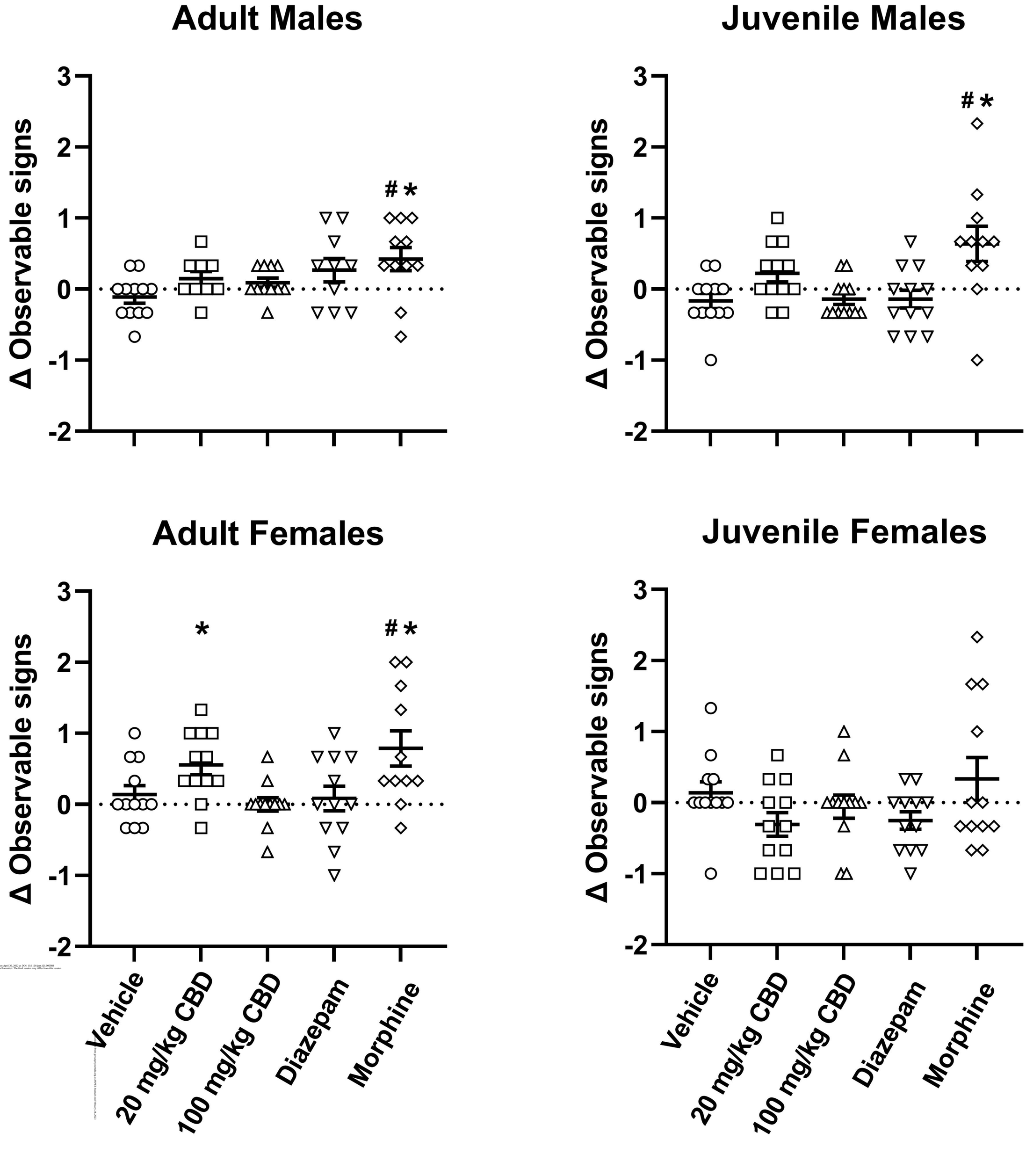

Adult Males
Juvenile Males
Adult Females
Juvenile Females
Δ Weight (g)
Δ Food comsuption (g)
Δ Temp (°C)
Vehicle
20 mg/kg CBD
100 mg/kg CBD
Diazepam
Morphine

Figure 5



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