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Cannabidiol reduces withdrawal symptoms in nicotine dependent rats

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Author Contributions

MK, OG, KH, and JD designed the experiment; LCS and LT performed the behavioral experiments, nicotine and cotinine blood extraction. LCS and MK analyzed the data and prepared the manuscript and figures. YS performed LCMS analysis of nicotine and cotinine serum levels, JDM supervised and interpreted nicotine and cotinine serum level determinations; RS, MH, and RF developed the isotope dilution LC/MS/MS assay for quantifying CBD in blood specimens; MK and OG edited the manuscript and figures. All authors approve the final version of the manuscript.

Abstract

Rationale: Cannabidiol (CBD) reduces craving in animal models of alcohol and cocaine seeking and is known to modulate nicotinic receptor function, suggesting that it may alleviate symptoms of nicotine withdrawal; however, preclinical evaluation of its efficacy is still lacking.

Objectives: The goal of this study was to test the preclinical efficacy of a chronic CBD treatment in reducing nicotine dependence using measures of withdrawal symptoms including somatic signs, hyperalgesia, and weight gain during acute and protracted abstinence.

Methods: Male and female Wistar rats were made dependent on nicotine using osmotic minipumps (3.15 mg/kg/day) for two weeks, after which minipumps were removed to induce spontaneous withdrawal. Three groups received CBD injections at doses of 7.5, 15, and 30 mg/kg/day for two weeks, starting one week into chronic nicotine infusion. The control groups included rats with nicotine minipumps that received vehicle injections of sesame oil instead of CBD; rats implanted with saline minipumps that received sesame oil injections (double vehicle) or the highest dose of CBD 30mg/kg/day. Throughout the experiment, serum was collected for determination of CBD and nicotine concentrations, mechanical sensitivity threshold and withdrawal scores were measured, and body weight was recorded.

Results: CBD prevented rats from exhibiting somatic signs of withdrawal and hyperalgesia during acute and protracted abstinence. There was no dose-response observed for CBD, suggesting a ceiling effect at the doses used and the potential for lower effective doses of CBD. The saline minipump group did not show either somatic signs of withdrawal or hyperalgesia during acute and protracted abstinence, and the highest dose of CBD used (30mg/kg/day) did not alter these results.

Conclusions: This preclinical study suggests that using CBD as a strategy to alleviate the withdrawal symptoms upon nicotine-cessation may be beneficial.

Keywords: CBD, tobacco, addiction, treatment, abstinence, withdrawal

Introduction

Nicotine abuse through tobacco smoking is the leading cause of preventable death in the United States, with an annual loss of life of about 500,000 (Warren, Alberg et al. 2014). Despite the declining rates of combustible cigarette smoking in the last 50 years, the use of electronic cigarettes containing nicotine is drastically increasing (Warren, Alberg et al. 2014). In addition to the rise in electronic cigarette use, the popularity of oral smokeless tobacco products, such as chewable tobacco, or "snuff," remains high in some parts of the world, such as South East Asia (Khan, Farooq et al. 2020).

To date, the most effective pharmacological approach to smoking cessation is the administration of varenicline (Chantix), a nicotinic acetylcholine receptor agonist, which has a ~23% cessation rate in clinical studies (Prochaska and Benowitz 2016) (Stitzer and Gross 1988). Nicotine withdrawal is characterized by somatic (tremors, bradycardia, gastrointestinal discomfort, sleep disturbances, and increased appetite), affective (craving, insomnia, anxiety, anhedonia, depression, dysphoria, hyperalgesia, and irritability) and cognitive symptoms (Stitzer and Gross 1988, Cohen, Pickworth et al. 1991, McLaughlin, Dani et al. 2015). Other methods of cessation approved by the U.S. Food and Drug Administration (FDA) include nicotine replacement therapy and bupropion (Prochaska and Benowitz 2016).

Cannabidiol (CBD) is one of the phytocannabinoids found in the cannabis plant and is believed to be non-psychoactive while producing anxiolytic, antipsychotic, and neuroprotective, effects (Alvarez, Lafuente et al. 2008, Zuardi 2008). CBD also decreases neuronal hyperexcitability in epilepsy, and in 2018, the FDA approved the use of Epidiolex, a CBD oil extract administered orally, for the treatment of Dravet and Lennox-Gastaut syndromes, two rare forms of epilepsy (2018). In both epilepsy and nicotine withdrawal, there is prominent neuronal hyperexcitability, albeit lower with withdrawal, suggesting that CBD may potentially improve withdrawal symptoms. CBD has also been found to modulate the nicotinic, opioid, serotonin, and endocannabinoid systems (Russo, Burnett et al. 2005, Kathmann, Flau et al. 2006, Thomas, Baillie et al. 2007). When nicotine dependent rats undergo spontaneous withdrawal and protracted abstinence, they exhibit dysregulation of endocannabinoids in the amygdala, hypothalamus, and hippocampus (Cippitelli, Astarita et al. 2011). Several studies have shown that chronic administration of cannabinoid receptor modulators like CBD can alter endocannabinoid receptor levels in the central nervous system (Gonzalez, Cebeira et al. 2005). Furthermore, CBD has been found to inhibit the $\alpha 7$ nicotinic acetylcholine receptor (Mahgoub, Keun-Hang et al. 2013), which plays a role in the reinforcing effects of nicotine (Markou and Paterson 2001). Interactions of endocannabinoids with the CB_1 receptor are essential in mediating behaviors associated with addiction (Sloan, Gowin et al. 2017). Finally, chronic CBD treatment prevents relapse to cocaine and alcohol-seeking in rodents (Liput, Hammell et al. 2013, Gonzalez-Cuevas, Martin-Fardon et al. 2018, Viudez-Martinez, Garcia-Gutierrez et al. 2018), and there is preliminary clinical observation suggesting that CBD may help reduce the number of cigarettes smoked in a day (Morgan, Das et al. 2013).

However, there has been no evaluation of CBD in a preclinical model of nicotine dependence, so here we sought to test the effect of chronic CBD treatment on somatic signs of withdrawal, hyperalgesia, weight, and nicotine metabolite levels. Rats made dependent on nicotine using subcutaneous minipumps (3.15 mg/kg/day) were treated daily with CBD for two weeks: during the last week of nicotine exposure, and the first week of abstinence. Rats were tested for symptoms of acute and protracted withdrawal upon nicotine cessation, including physical signs of withdrawal, hyperalgesia, and body weight gain. Varying doses of CBD (0, 7.5, 15, and 30 mg/kg/day) were tested to identify the lowest effective dose and blood levels of CBD, nicotine, and cotinine were examined throughout the study.

Materials and Methods

Animals

Wistar rats (*n* = 84 [42 males, 42 females]) weighing 250-275g (Charles River) were purchased at seven weeks old. Rats were housed two per cage with (Teklad 7090, Lab-Grade Sani-chios, Envigo, WI) bedding and maintained on a 12/12h light/dark cycle with *ad libitum* access to (Teklad 8604, Lab Rodent Diet, Envigo, WI) food and tap water. Rats were handled for ten minutes daily for three days by the experimenter. All the procedures were performed two hours into the dark cycle and were approved by the University of California, San Diego Institutional Animal Care and Use Committee.

Drugs

Nicotine hydrogen tartrate (Sigma) was dissolved in saline (nicotine dose 3.15 mg/kg/day) and brought to pH 7.4 with NaOH 1M. Osmotic minipumps (Alzet AP 2ML2) were

filled with 2 ml of the nicotine solution the night before surgery was performed. Cannabidiol (Noramco Inc) was dissolved in sesame oil (Sigma) for daily subcutaneous injection at the doses 0, 7.5, 15, and 30 mg/kg. The higher dose of CBD used (30 mg/kg) translates to ~200 mg/kg in humans, which is recommended for CBD treatment of epilepsy (PMID: 30681657). Other groups have reported successful results of CBD at doses 15 and 30 mg/kg in anxiety like behavior in rats (PMID: 29686308) Treatment with CBD was performed for 14 consecutive days using sesame oil (Sigma) as a control. CBD was administered daily starting 7 days after minipump implantation. On test days, CBD was administered 3 hours prior to the behavioral assessments.

Osmotic Minipump Implantation

Rats were anesthetized with 3% isoflurane in O_2. An incision was made in the lumbar region, and the peritoneal cavity was opened to fit the minipump (Alzet). The incision was closed with three sutures and surgical glue. The osmotic minipumps were filled with nicotine solution or saline. The concentration of nicotine was adjusted for rat weight and minipump flow rate to deliver nicotine at 3.15 mg/kg/day for 14 days.

1: Effect of CBD on nicotine-induced somatic signs of withdrawal

Somatic signs of withdrawal were measured during acute (24 h) and protracted (7 days) nicotine or saline induced withdrawal, immediately following the mechanical sensitivity assay, see Fig. 1A and Fig. 5A for timeline. Somatic signs of withdrawal such as: jumps, teeth chattering, ptosis, blinks, head shakes, paw tremors, abdominal contractions, genital licks, yawns, were observed for 30 minutes and recorded for each rat (Malin, Lake et al. 1992). The

total number of observed behaviors is reported as the withdrawal score, with a higher score indicating more withdrawal behavior.

2: Effect of CBD on nicotine-induced hyperalgesia

Paw withdrawal in response to a dynamic plantar aesthesiometer (Ugo Basile) was tested for mechanical sensitivity as adapted from previous reports (Chaplan, Bach et al. 1994). Time of paw retraction and force exercised to produce a paw retraction was recorded for three measures for each back paw. Measurements were obtained at baseline, during nicotine or saline infusion, acute withdrawal, and protracted abstinence (see Fig. 2A and Fig. 5A for timeline). Data are expressed as % change vs. baseline for each individual to allow for a within-individual evaluation of hyperalgesia.

3: Effect of CBD on nicotine withdrawal-induced weight gain

Bodyweight was measured at the baseline, nicotine or saline minipump infusion, acute withdrawal, and protracted abstinence timepoints. See Fig. 3A and Fig 5A for timeline. The data at the acute withdrawal and protracted abstinence timepoints were analyzed based on the percentage change vs. the nicotine/saline minipump timepoint for each individual to allow for within-individual evaluation of weight gain induced by nicotine cessation.

4: CBD, Cotinine, and Nicotine Serum Levels

Blood (300 μL) was collected at baseline, during nicotine or saline infusion, acute withdrawal, and protracted abstinence. To ensure maximum cannabidiol extraction and detection, only dose 30 mg/ml CBD was collected and analyzed, see Fig. 4A and Fig. 5A for

timeline. Blood was allowed to clot for 30 minutes at room temperature spun at 13,000 RPM for 10 minutes, and serum (100 μL) was collected for processing. Nicotine, cotinine, and CBD levels were analyzed by tandem liquid chromatography-mass spectrometry as previously described (Sobolesky, Smith et al. 2019, Hubbard, Smith et al. 2020). Briefly, nicotine and cotinine were extracted from sera (20 μL) with acetonitrile (60 μL), containing 100 ng/ml D3-nicotine and 300 ng/ml D3-cotinine internal standards. CBD was extracted from sera (60 μL) with an extraction buffer containing 100 ng/ml D3-cannabidiol internal standard. Samples for CBD analysis were then followed by solid-phase extraction using Oasis Prime HLB C18 SPE plates, as previously described (Sobolesky, Smith et al. 2019).

Statistical Analysis

Statistics were calculated using GraphPad Prism 8.0. All behavioral data were analyzed using a one-way analysis of variance (ANOVA). Weight gain was analyzed using a two-way analysis of variance (2-way ANOVA). Significant effects in the ANOVA were followed by the Newman–Keuls *post hoc* test. Blood levels were analyzed using a two-tailed t-test. The data in the control saline minipump and CBD or its vehicle injection shown in Fig. 5, were analyzed using a two-tailed t-test. All values are expressed as mean ± SEM. Significant outliers were removed when appropriate using the Grubb's outlier test. The data were analyzed for sex differences, however, there was no significant effect of sex in any experiment. Therefore, the data are presented as males and females combined.

Results

1: Effect of CBD on nicotine-induced somatic signs of withdrawal

To test the effect of CBD on nicotine cessation-induced somatic signs of withdrawal, animals were observed for 30 min, and their withdrawal behaviors were scored, see Fig. 1A for the experimental timeline. Statistical analysis one-way ANOVA showed a significant group effect during acute nicotine withdrawal (one-way ANOVA, $F_{4,55} = 3.126$, $p < 0.05$; Fig. 1B). The Newman-Keuls multiple-comparison test revealed a significant increase in somatic signs of withdrawal from the vehicle group to the CBD 0 group ($p<0.05$). This group effect was sustained into protracted abstinence (one-way ANOVA, $F_{4,55} = 5.806$, $p < 0.001$; Fig. 1C). The Newman-Keuls multiple-comparison test revealed a significant increase in somatic signs from the vehicle to the nicotine group ($p<0.001$) and a significant difference between the nicotine and CBD groups ($p<0.05$). In a control experiment, we tested the effect of CBD 30mg/kg on saline cessation-induced somatic signs of withdrawal, see Fig. 5A for the experimental timeline. CBD 30mg/kg did not affect the behavior of rats after 24 h ($t_{11} = 0.785$, $p>0.05$; Fig. 5B) or after 7 days ($t_{11} = 0.512$, $p>0.05$; Fig. 5C) of saline cessation.

2: Effect of CBD on nicotine-induced hyperalgesia

To test the effect of CBD on nicotine cessation-induced hyperalgesia, an aesthesiometer was used to record paw withdrawal and associated pain threshold automatically. The data are presented as the percent of baseline pain threshold, see Fig. 2A for the experimental timeline. We did not observe any group effect during acute nicotine withdrawal, as shown from the one-way ANOVA analysis ($F_{4,50} = 2.187$, $p > 0.05$; Fig. 1B). However, during protracted nicotine withdrawal a significant group effect emerged (one-way ANOVA, $F_{4,51} = 4.066$, $p < 0.01$; Fig. 1C). The Newman-Keuls multiple-comparison test revealed a significant decrease in pain threshold from the vehicle to the CBD 0 group ($p<0.05$) and a significant difference between the

nicotine and CBD 7.5 and CBD 15 groups (p<0.05). To test the effect of CBD on any potential saline cessation-induced hyperalgesia, we analyzed the data as previously described. Student's *t*-test analysis did not reveal any statistically significant difference between the saline and the CBD treated rats in acute withdrawal (t_{11} = 0.932, p>0.05; Fig. 5D) and in protracted abstinence (t_{11} = 0.107, p>0.05; Fig. 5E).

3: Effect of CBD on nicotine withdrawal-induced weight gain

To test the effect of CBD on nicotine cessation-induced weight gain, weight was measured throughout the experiment, and the weight change during the acute withdrawal and protracted abstinence timepoints were analyzed as the percent of the weight during the minipump timepoint (Fig. 3A). A significant effect of time was observed from the minipump to the withdrawal time point (2-way ANOVA, $F_{1,55}$ = 18.62, p <0.0001; Fig. 3B). The Newman-Keuls multiple-comparison test revealed a significant difference in weight gain of the CBD 0 group from the nicotine minipump timepoint to the acute withdrawal timepoint (7 days, p<0.01). A significant effect of time was also observed from the acute withdrawal to the protracted abstinence time point (2-way ANOVA, $F_{1,55}$ = 92.77, p <0.0001; Fig. 3C). The Newman-Keuls multiple-comparison test revealed a significant difference in weight gain of the CBD 0, 7.5, 15, and 30 groups from the acute withdrawal to the protracted abstinence timepoint (7 days, p<0.05 for CBD 0, p<0.0001 for CBD 7.5, 15, and 30). Additionally, there was a significant interaction of time and treatment from the acute withdrawal to the protracted abstinence time point (2-way ANOVA, $F_{4,55}$ = 3.143, p <0.05; Fig. 3C). Student's t-test analysis revealed a significant increase in the body weight of rats implanted with saline minipump and injected daily with the CBD vehicle (t_{11} = 2.66, p<0.05; Fig. 5F) from the acute to the protracted abstinence time point. No

differences were observed in the group of rats implanted with saline minipump and injected daily with the CBD 30mg/kg/day ($t_{11} = 2.66$, $p<0.05$; Fig. 5F)

3: CBD and Nicotine Serum Levels

To test the effect of CBD on the pharmacokinetics of nicotine, levels of nicotine, cotinine, and CBD were measured in serum throughout the experiment, see Fig. 4A for timeline. Nicotine levels were consistent at 65-70 ng/ml between groups during the minipump exposure period (students *t*-test, $t_{10} = 0.4939$, $p>0.05$; Fig. 4B). Cotinine levels were significantly different between the nicotine and the CBD 30 group after the first injection of CBD (students *t*-test, $t_{10} = 2.357$, $p<0.05$; Fig. 4C). Since we did not observe a dose-response in the behavioral data, only the highest dose of CBD was measured for pharmacokinetics. The half-life of CBD was determined to be ~35 h, with an area under the curve indicating ~1000 ng*h/ml CBD from a single sesame oil injection at a dose of 30 mg/kg (Fig. 4D). CBD levels were consistent at 180-220 ng/ml within the CBD30 group throughout the administration period (students *t*-test, $t_{21} = 1.482$, $p>0.05$; Fig. 4E). In a small sample of rats implanted with saline minipumps (n=5/group), we analyzed whether the levels of CBD were consistent during acute and protracted withdrawal. Student *t*-test analysis did not show any statistically significant difference in the levels of CBD in the serum in both timepoints ($t_4 = 1.4$, $p>0.05$; Fig. 5G)

Discussion

This study provides results demonstrating the efficacy of CBD in reducing symptoms of nicotine withdrawal in rats. Daily treatment with CBD (7.5, 15, and 30 mg/kg) for two weeks prevented the somatic signs of withdrawal-induced by nicotine during acute and protracted

abstinence in rats (Fig. 1). Hyperalgesia was attenuated by CBD (7.5 and 15 mg/kg) during protracted abstinence to nicotine, and there was a similar trend during acute withdrawal (Fig. 2). Weight gain during acute withdrawal was only observed for the CBD 0 group, however, during protracted abstinence, all nicotine-dependent groups showed significant weight gain with or without CBD treatment (0, 7.5, 15, and 30 mg/kg; Fig. 3). CBD (30 mg/kg) reduced the levels of sera cotinine during the nicotine minipump timepoint (Fig. 4). Importantly, CBD (30 mg/kg) did not affect the behavior of the saline minipump implanted rats (Fig. 5). Together these results suggest promise for CBD in preventing or reducing the symptoms of withdrawal following nicotine cessation.

Nicotine withdrawal syndrome is typically characterized by somatic signs of withdrawal in humans and rodents (Cohen, Pickworth et al. 1991, Malin, Lake et al. 1992, Damaj, Kao et al. 2003). Symptoms may start as soon as hours into cessation and last up to several weeks (McLaughlin, Dani et al. 2015). Nicotine-dependent rats exhibited somatic signs of withdrawal 24 h after cessation, lasting up to one week into nicotine abstinence. This result is consistent with a vast literature using nicotine-minipump in rodents to produce nicotine dependence. The somatic signs of withdrawal in rats (i.e., ptosis, head shakes, and abdominal constrictions) have relevant predictive validity for tobacco withdrawal syndrome in humans (Cohen, Pickworth et al. 1991, Malin, Lake et al. 1992, Damaj, Kao et al. 2003). For instance, somatic signs of withdrawal in rodents and withdrawal signs in humans are both reduced by the administration of FDA-approved medications such as nicotine replacement therapy, varenicline, and bupropion (Mooney and Sofuoglu 2006, West, Baker et al. 2008, Igari, Alexander et al. 2014). In humans, symptoms may start as soon as hours into cessation and last up to months, while, as in the current

study, the somatic signs of withdrawal in rodents usually last 3-7 days (McLaughlin, Dani et al. 2015). Daily treatment with CBD prevented the somatic signs of withdrawal at all doses tested. The effect size observed with the highest dose of CBD (Cohen's d = 1.6, 30 mg/kg/day) is similar to effect size observed after varenicline and bupropion (Cohen's d ~ 1.9 and ~ 1.5, respectively) during behavioral measurements of withdrawal in rodents (Cryan, Bruijnzeel et al. 2003, Malin, Lake et al. 2006, Damaj, Grabus et al. 2010, Jackson, Silk et al. 2017, Bagdas, Alkhlaif et al. 2018). This comparison raises the possibility that CBD may produce similar therapeutic efficacy with fewer side effects, as CBD is generally well tolerated with low risk of serious adverse events or dependence (Viudez-Martinez, Garcia-Gutierrez et al. 2019, Larsen and Shahinas 2020).

Mechanical allodynia is a robust method for measuring hyperalgesia during nicotine withdrawal (Carstens, Anderson et al. 2001, Cohen, Treweek et al. 2015, Hamouda, Jackson et al. 2018, Pahng and Edwards 2018, Xue, Kallupi et al. 2018). In the present study, a reduction of hyperalgesia was observed at all the doses of CBD tested during acute withdrawal and protracted abstinence. This reversal is similar to that observed with desformylflustrabromine, an allosteric modulator of the $\alpha4\beta2$ nicotinic acetylcholine receptor, and a nicotine degrading enzyme in the same model of nicotine dependence (Hamouda, Jackson et al. 2018, Xue, Kallupi et al. 2018). Increased tactile sensitivity and hyperalgesia are typically associated with nicotine withdrawal syndrome in humans and rats and can last weeks into abstinence (Chaplan, Bach et al. 1994, Carstens, Anderson et al. 2001). A potential caveat is that we did not observe a dose-response with CBD at the doses tested here. One explanation may be that a ceiling effect was

reached and that lower doses of CBD may be effective for alleviating the symptoms of nicotine withdrawal. Further studies are needed to investigate the impact of lower doses of CBD.

Weight gain is typically seen during nicotine withdrawal in animals and humans (Leischow and Stitzer 1991, Perkins 1993, Malin and Goyarzu 2009). In the current study, weight gain between minipump and the acute withdrawal time point was only observed in the CBD 0 group. This suggests that CBD treatment may limit gain weight during transition from nicotine taking to cessation. All of the nicotine-dependent groups showed further weight gain from the acute withdrawal to the protracted abstinence timepoint, suggesting CBD alone may not be useful in preventing long-term weight gain during nicotine abstinence. However, a combination of nicotine replacement therapy and CBD strategy may be useful in preventing nicotine withdrawal-induced weight gain.

Nicotine dependence induced by chronic infusion via osmotic minipumps is known to be a robust method to produce dependence and is associated with nicotine blood levels in the 50-100 ng/ml range and levels of cotinine in the 300-900 ng/ml range. These results fall well into the typical range and are similar to our previous studies (Shoaib and Stolerman 1999, Xue, Kallupi et al. 2018). Such nicotine (50-100 ng/ml) and cotinine (300-900 ng/ml) blood levels are also similar to the levels observed in smokers (Russell, Feyerabend et al. 1976, Gourlay and Benowitz 1997, Hiler, Breland et al. 2017). The levels of CBD throughout the behavioral testing timepoints (~300 ng/ml) are on par with prior reports in rats using a similar dose (Gonzalez-Cuevas, Martin-Fardon et al. 2018). We found a slight decrease (27%) in blood cotinine levels in animals treated with CBD (30mg/kg) without changes in nicotine levels. These results suggest

that the effect of CBD on nicotine metabolite levels during exposure to nicotine may have contributed to its effect on reducing withdrawal symptoms (Anderson and Chan 2016), however further studies are necessary to test this hypothesis. There are several potential interactions of nicotine and CBD that could result in altered metabolism that need to be investigated. Tobacco and marijuana smoking both induce the cytochrome P450 enzyme CYP1A2, nicotine is metabolized into cotinine by CYP2A6, CBD is primarily metabolized by CYP3A4, and CBD may inhibit CYP2C19 (Stout and Cimino 2014, Anderson and Chan 2016).

Limitations

We did not observe a dose-effect of CBD at the doses tested here, suggesting a ceiling effect and the potential for a lower effective dose.

CBD does not activate CB_1 and CB_2 receptors. It is known to be a "multitarget" drug. At very low concentrations, CBD blocks, among other the orphan G-protein-coupled receptor GPR55, that is thought to play a role in the central nervous system and enhances the activity of the 5-HT_{1a} receptor. At higher concentrations, CBD activates the nuclear peroxisome proliferator-activated receptor-γ, that plays a major regulatory role in energy homeostasis and metabolic function (PMID: 24854329). Future studies with lower doses of CBD need to be performed.

We also did not observe sex differences in the effect of CBD on the symptoms of nicotine dependence, however, this could be due to the small sample size. Future studies should investigate the effects of CBD on nicotine dependence in males and females with higher statistical power. While there may be concern about potential adverse consequences when administered to those recovering from addiction, preliminary clinical studies have shown minimal adverse effects of CBD on memory and no adverse effects on impulsivity during

abstinence in cigarette dependent smokers (Hindocha, Freeman et al. 2018). Furthermore, CBD was found to reverse the salience and pleasantness of cigarette cues during acute abstinence in dependent smokers, with no subjective side-effects (Hindocha, Freeman et al. 2018). In the present study we determined the effect of CBD on nicotine and cotinine levels after a single CBD administration, future studies should determine the effect of CBD on nicotine metabolism after long-term treatment.

Conclusion

This study provides preclinical evidence of therapeutic efficacy of chronic CBD treatment on nicotine withdrawal syndrome, with a reduction of somatic signs and hyperalgesia. Further work on the efficacy of CBD in reducing intake, motivation and preventing relapse in nicotine dependent animals in a self-administration paradigm is necessary.

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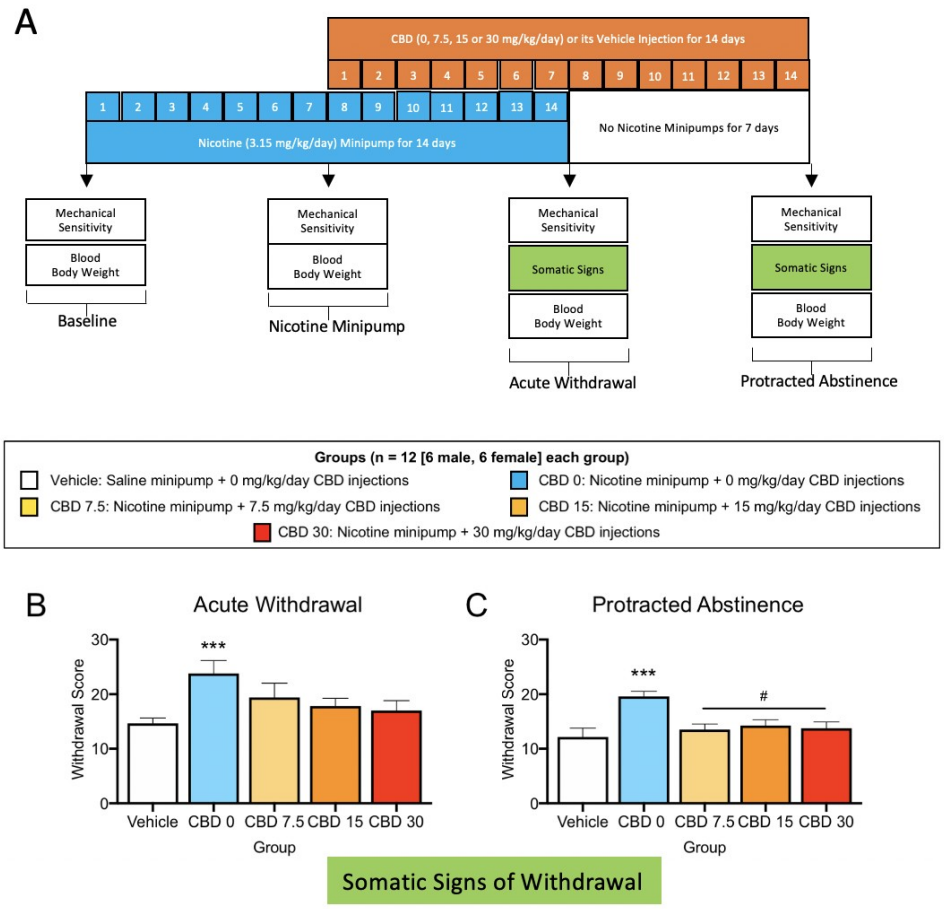

Figure 1. CBD reduces nicotine cessation-induced somatic signs of withdrawal. A) Experimental timeline and group design; B) Somatic signs of withdrawal during acute withdrawal. C) Somatic signs of withdrawal during protracted abstinence (***$p < 0.001$, from vehicle, #$p < 0.05$ from CBD 0). Error bars represent the SEM of the mean (n = 12 [6 male, 6 female]).

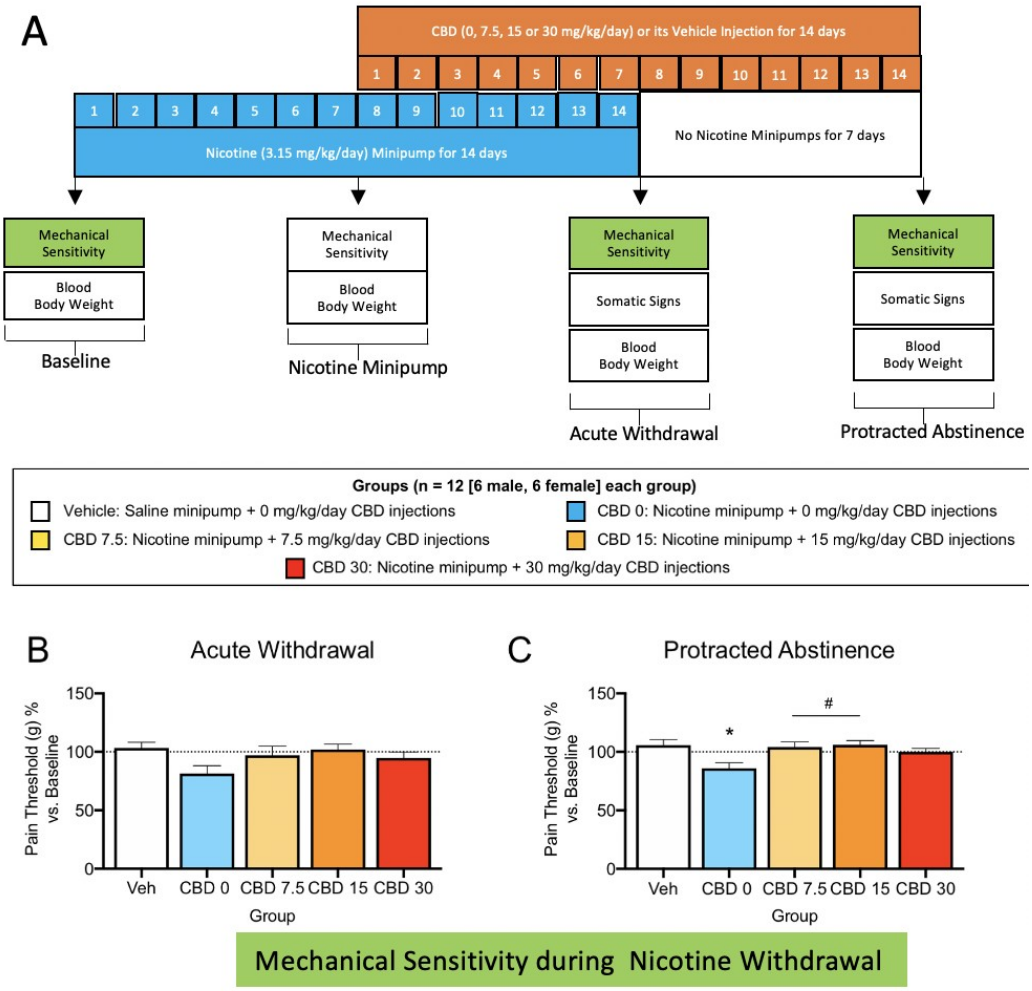

Figure 2. CBD prevents nicotine cessation-induced hyperalgesia. A) Experimental timeline and group design; B) Pain threshold as percent (g) of baseline during acute withdrawal. C) Pain threshold as percent (g) of baseline during protracted abstinence (*$p < 0.001$, from vehicle, #$p < 0.05$ from CBD 0). Error bars represent the SEM of the mean (n = 12 [6 male, 6 female]).

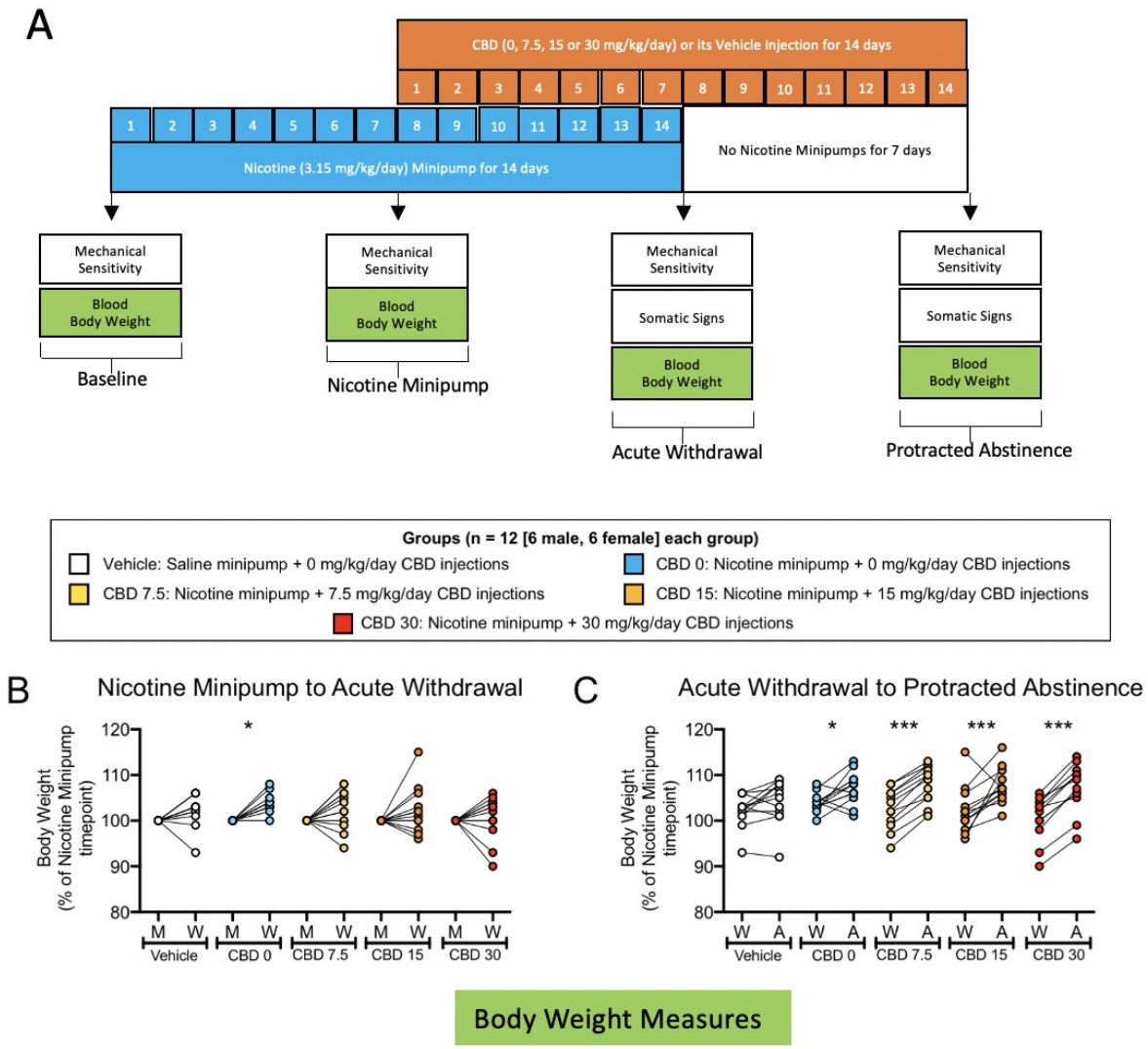

Figure 3. CBD prevents weight gain during acute withdrawal but not protracted abstinence. A) Experimental timeline and group design; B) Body weight as percent (g) of nicotine minipump timepoint from the minipump (M) to acute withdrawal (W) timepoints (*p < 0.05, from vehicle). C) Body weight as percent (g) of nicotine minipump timepoint from the acute withdrawal (W) to the protracted abstinence (A) timepoints (*p < 0.05 and ***p < 0.001, from vehicle). Data is represented as individual rats (n = 12 [6 male, 6 female]).

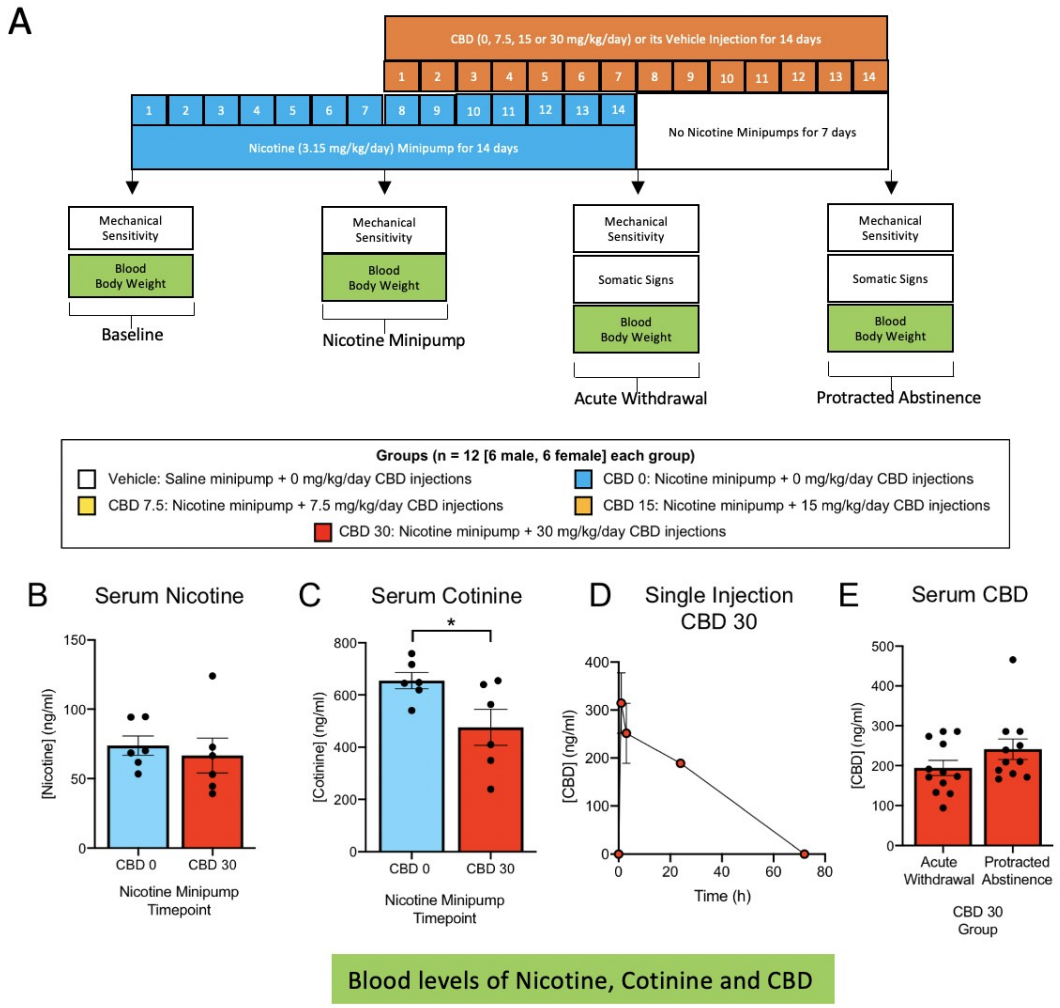

Figure 4. CBD reduces serum nicotine metabolite levels. A) Experimental timeline and group design; B) Serum nicotine concentration during the nicotine minipump timepoint for the CBD 0 and CBD 30 groups. C) Serum cotinine concentration during the nicotine minipump timepoint for the CBD 0 and CBD 30 groups (*$p < 0.05$). Error bars in B and C represent the SEM of the mean ($n = 6$ [3 male, 3 female]); D) Serum CBD concentration time course after a single injection (s.c.) of 30 mg/kg CBD in sesame oil. Error bars represent the SEM of the mean ($n = 3$ [1 male, 2 female]); E) Serum CBD concentration for the CBD 30 group during the acute

withdrawal and protracted abstinence timepoints. Error bars represent the SEM of the mean (n = 12 [6 male, 6 female]).

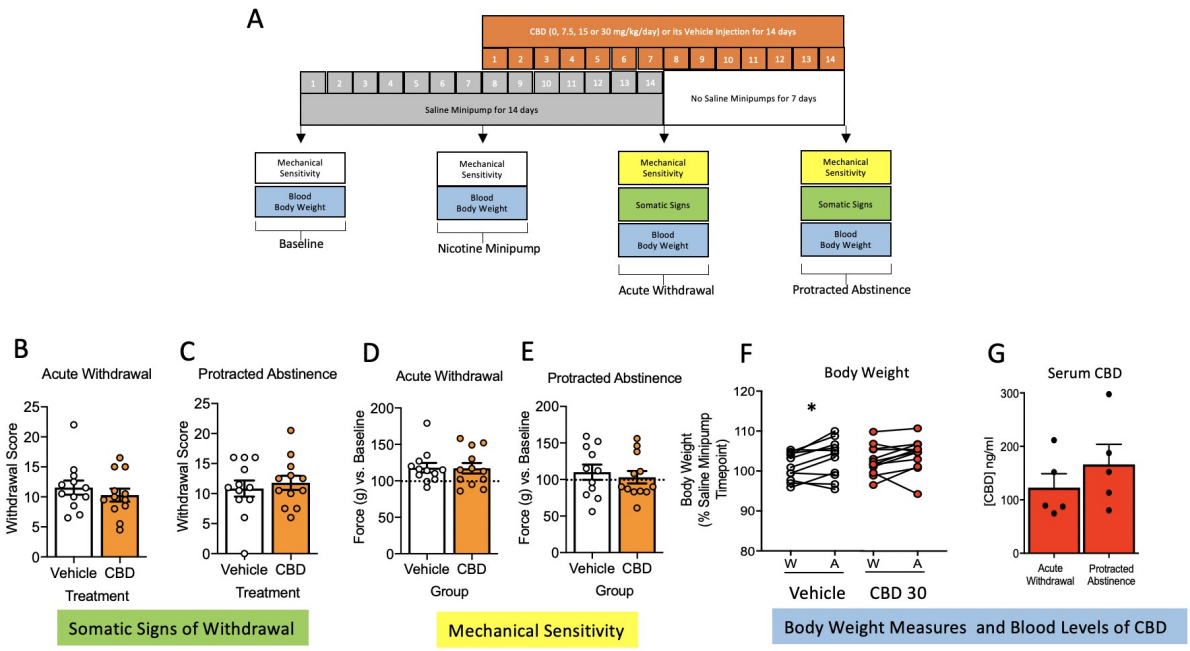

Figure 5. CBD does not alter the behavior in the saline minipump group. A) Experimental timeline and group design; B) Somatic signs of withdrawal during acute withdrawal. C) Somatic signs of withdrawal during protracted abstinence. D) Pain threshold as percent (g) of baseline during acute withdrawal. C) Pain threshold as percent (g) of baseline during protracted abstinence. F) Body weight as percent (g) of nicotine minipump timepoint from the acute withdrawal (W) to the protracted abstinence (A) timepoints (*$p < 0.05$). All data is represented as individual rats (n = 12 [6 male, 6 female]). E) Serum CBD concentration for the CBD 30 group during the acute withdrawal and protracted abstinence timepoints. Error bars represent the SEM of the mean (n = 5 [3 male, 2 female]).