

Therapeutic-like activity of cannabidiolic acid methyl ester (HU-580) in the MK-801 mouse model of schizophrenia: role for cannabinoid CB1 and serotonin-1A receptors

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Abstract

Schizophrenia is an incurable psychotic illness. Those diagnosed have limited pharmacological treatment options, many of which do not provide long term relief and come with unpleasant side effects. The endocannabinoid and serotonergic systems are important neuromodulators in psychotic illness. We hypothesize that cannabidiolic acid methyl ester (HU-580) that exerts action on both these systems could have therapeutic potential by antagonizing cannabinoid receptor-1 (CB1R) and agonizing 5-hydroxytryptamine receptor-1A (5-HT1AR). We employed behavioural and brain protein analyses in male and female mice exposed to MK-801, which precipitated schizophrenia-related reactivity across a number of behavioural dimensions. C57BL/6 mice were subjected to a battery of behavioral tests, and we found that subchronic treatment of MK-801 (once daily for seven days) induced positive-like, negative-like, and cognitive-related behavioral deficits; primarily in females. Sub-chronic treatment of MK-801 (once daily for 17 days) induced positive- and negative-like behavioral deficits in females. Low-dose (0.01ug/kg) but not high-dose (0.05 ug/kg) treatment rescued female mice from schizophrenia-related behavioral deficits. Altogether, these data suggest that HU-580 may have dose-dependent antipsychotic-like potential that rely on mechanisms that recruit CB1R and 5-HT1AR.

Introduction

Schizophrenia is a heterogenous psychotic disorder that remains incurable. There are roughly over 21 million people living with schizophrenia, a prevalence that has been increasing over the last two decades (Charlson et al., 2018). Schizophrenia has multiple symptom presentations that typically emerge in late adolescence and early adulthood. These include positive (e.g., psychosis and delusions), negative (e.g., anhedonia and flat affect), and cognitive (e.g., memory deficits) symptoms (Marder & Galderisi, 2017; Marzouk et al., 2020; Bowie & Harvey, 2006). Current treatment comes with unpleasant extrapyramidal side effects and may not provide long term relief (Lally & MacCabe, 2015). Negative symptoms are more strongly correlated with poorer baseline functioning (Rabinowitz et al., 2012; Leifker, Bowie, & Harvey, 2009), and appear to be resistant against current antipsychotic treatment (Hanson et al., 2010; Lin, Tsai, & Lane, 2014). Thus, new alternative treatments are needed that are better tolerated and continue to provide relief.

Glutamate influences the development of schizophrenia through the N-methyl-D-aspartate receptor (NMDAR) system. NMDAR antagonists are frequently used as models of schizophrenia in animal research, and symptoms induced in rodents are comparable to clinical schizophrenia (Krystal et al., 1994; Javitt & Zukin, 1991). Dizocilpine (MK-801) is an NMDAR antagonist often used to model schizophrenia and has been shown to induce a number of behaviors in rodents that are indicative of schizophrenia-like behavior such as increased locomotor activity, changes in locomotor activity in response to stress, pre-pulse inhibition deficits,

impaired spatial memory, and social withdrawal (Bubeníková-Valešová et al., 2008), and similar behaviors are seen in humans (Seillier & Giuffrida, 2009; Bubeníková-Valešová et al., 2008).

The neuro-modulatory serotonergic and endocannabinoid systems have been implicated in psychotic illness as potential therapeutic pathways (Davies & Bhattacharyya, 2019; Yang & Tsai, 2017). Serotonin is implicated in schizophrenia because it is a primary regulator of dopamine and second-generation antipsychotics antagonize 5-hydroxytryptamine receptor 2A (5-HT_{2A}) to provide relief for negative symptoms more so than first-generation antipsychotics (Kim, 2021; Ohno, 2011). Indeed, drugs that are 5-hydroxytryptamine receptor 1A (5-HT_{1A}) agonist provide relief for positive and negative symptoms without extrapyramidal side effects (Lobo et al., 2022). Moreover, increased 5-HT_{1A} receptor density is seen in schizophrenic patients, and may be sensitized due to overstimulating serotonin 5-HT_{2A} receptors (Kim, 2021). Dopaminergic and serotonergic neurons can communicate with the endocannabinoid system to regulate excitability within the brain via glutamate and γ -aminobutyric acid (GABA; Peters, Cheer, & Tonini, 2021), therefore there are complex crosstalk relationships in the regulation of glutamate activity. Agonizing cannabinoid receptor type 1 (CB₁R) results in increased glutamate and dopamine and increases locomotive activity consistent in schizophrenic-like behavior (Polissidis et al., 2013). Conversely, antagonizing CB₁R results in symptom relief (Ballmaier et al., 2007).

This study tested deficits induced by MK-801 in mice and investigate if cannabidiolic acid (CBDA) methyl ester (HU-580) would provide therapeutic relief. HU-580 is a more stable CBDA analogue that is shown to be a strong agonist of 5-HT_{1A} and an antagonist of CB₁R (Pertwee et al., 2018; Navarro et al., 2020). We have ascertained that exposure to MK-801 would induce schizophrenic-like behaviors and pathophysiology that would be attenuated by HU-580.

Experimental procedures

Animals

This experiment utilized adult C57BL/6 mice (Charles-River Saint-Constant, PQ, Canada) single housed weighing 25 – 35g. Experiments were conducted between 8:00 and 20:00. All procedures were in accordance with the ethical guidelines by Memorial University’s institutional animal care and use committee and the Canadian Institutes of Health Research. Mice were pseudo-randomized into two factors, exposure (control or MK-801, n = 30 per factor, 15 males and 15 females) and treatment (HU-580 - 0.01ug/kg, HU-580 - 0.05ug/kg, or vehicle; n = 20 per level, 10 males and 10 females), creating six groups: Ctrl+Veh, Ctrl+LowHU, Ctrl+HighHU, MK+Veh, MK+LowHU, and MK+HighHU (n = 10 per group, 5 males and 5 females).

Drugs

HU-580 was dissolved in vehicle that consisted of 5% Tween 80, 5% polyethylene glycol, and 90% saline (0.9%). MK-801 was dissolved in 0.9% saline. Drugs were purchased from Sigma-Aldrich. All drugs were administered intraperitoneally (i.p.).

MK-801 model of schizophrenia

The effect of MK-801 was tested as a model of schizophrenia. We assessed if MK-801 induced deficits consistent with schizophrenic-like behaviors and receptor sensitization. Mice were given saline (sal) or MK-801 (0.3mg/kg) for 7 days. Mice were then given MK-801 or saline and treatment (vehicle[veh], HU-580 0.01ug/kg, or HU-580 0.05 ug/kg) for 10 days. Behaviors were tested at one week and the end of the treatment phase.

HU-580 Treatment

On day 7, mice received HU-580 treatment or saline 30 minutes prior to behavioral tests. Following, mice continued co-treatment of MK-801 or saline and HU-580 or vehicle for ten days and were subjected to a second battery of behavioral tests.

Behavioral assay

Behavior was assessed using open field test (OFT), social interaction test (SIT), novel object recognition test (NORT) and forced swim test (FST). Behaviors were analyzed using an automated video tracking software (EthoVision) and/or Behavioral Observation Research Interactive Software (BORIS; Friard & Gamba, 2016). For manual scoring, raters were blind to mouse subject identification. Due to feasibility, testing order was maintained. Three identical testing chambers (24 x 54.5 x 40 cm³) were used and modified to fit each test, excluding FST. Chambers and all objects used in each test were cleaned with 70% ethanol between trials.

OFT: Hyperactivity, interpreted as psychomotor agitation, was assessed as total distance traveled in centimeters. Mice were placed in the chamber and were recorded for 10 minutes, consisting of 5 minutes of habituation and 5 minutes of behavioral analysis. *SIT*: To test spontaneous sociability and preference, Crawley's sociability test was used (Kaidanovich-Beilin et al., 2011). Spontaneous social behavior was measured in phase 1 by dividing time spent with a stranger conspecific with time spent with a stranger mouse to produce a social behavior index (SI). Social memory preference index (SMI) was measured in phase 2 by dividing time spent with a stranger conspecific with time spent with a familiar conspecific. *NORT*: In phase 1, two identical red plastic bulbs (2.5 x 2.5 cm³) were mounted at each end of the testing chambers using Velcro. Mice were placed in the center chamber, dividers were removed, and recording commenced for 5 minutes. In phase 2, one bulb was replaced with a gold bell or cone (2.5 x 2.5 cm³). *FST*: Mice were placed in a glass (8.5 x 20 cm) that was filled partway with water (22 – 25 degrees Celsius). A 2-minute period of habituation occurred before recording behavior for 4 minutes.

Enzyme-linked Immunosorbent Assay (ELISA)

Brains were flash frozen and stored in a -80 freezer. The PFC was removed was kept frozen throughout the entire process. The PFC was identified using a mouse brain atlas and visual landmarks. The tissue removed was within AP 2.93mm and 1.33mm. To prepare samples for ELISA, 1x phosphate-buffered saline (PBS) was micro-pipetted into the vials at a concentration of 10% tissue and 90% PBS as per recommendations of the kits. Samples were centrifuged at 1000 x g for 20 minutes and the supernatant was removed. Instructions were followed for each kit thoroughly. Each ELISA kit was purchased from MyBioSource, and this study tested for CB1R (Catalogue: MBS2533482) and 5-HT1AR (Catalogue: MBS287600).

Data analysis

Data were analyzed via SigmaPlot 15.0 using one-way or two-way ANOVA. Dunnett's post hoc analysis was used to control for experiment-wise error rate and to compare groups to control. Sexes were analyzed pooled and then separated to investigate sex differences. Data are shown as mean+/-standard error of the mean (SEM).

Results

Behavioral Measures

OFT: At 7 days, results revealed a significant interaction between exposure and treatment in females, $F(2,24) = 4.356, p = 0.024$. Post hoc comparisons revealed that MK+Sal (Mean[M] = 762.696, Standard Deviation[SD] = 123.944) was trending towards traveling significantly more than Ctrl+Sal (M = 600.930, SD = 160.251), MD = 161.765, $q = 1.683, p = 0.105$, and MK+LowHU (M = 498.434, SD = 211.761) was significantly less active than MK+Sal (M = 762.696, SD = 123.944), MD = 264.262, $q = 2.749, p = 0.021$. At 17 days, differences were significant, $F(5,54) = 7.065, p < 0.001$. Post hoc comparisons revealed that compared to Ctrl+Sal (M = 507.541, SD = 171.562), MK+Sal (M = 889.621, SD = 312.669), MD = 382.080, $q = 3.755, p = 0.002$, and MK+HighHU (M = 992.224, SD = 277.479), MD = 484.683, $q = 4.763, p < 0.001$, traveled significantly more. In males, results were significant, $F(5,24) = 7.843, p < 0.001$. Post hoc comparisons revealed that compared to Ctrl+Sal (M = 479.282, SD = 198.022), MK+HighHU (M = 1157.157, SD = 300.419) traveled significantly more, MD = 677.874, $q = 5127, p < 0.001$. In females, results were significant, $F(5,24) = 8.487, p < 0.001$. Post hoc comparisons revealed that MK+Sal (M = 1132.174, SD = 194.739) traveled significantly more than Ctrl+Sal (M = 535.799, SD = 158.165), MD =

596.375, $q = 5.761$, $p < 0.001$, Ctrl+LowHU (M = 668.763, SD = 83.481), MD = 463.411, $q = 4.477$, $p < 0.001$, Ctrl+HighHU (M = 712.275, SD = 96.458), MD = 419.899, $q = 4.057$, $p = 0.002$, MK+LowHU (M = 603.433, SD = 257.934), MD = 528.741, $q = 5.108$, $p < 0.001$, and MK+HighHU (M = 827.292, SD = 122.390), MD = 304.882, $q = 2.945$, $p = 0.029$ (See Figure 1).

SIT: At 7 days, results revealed a main effect of exposure on SI, $F(1,53) = 6.051$, $p = 0.017$. Post hoc comparisons revealed that MK+Sal mice (M = 1.845, SD = 0.22) had a significantly lower SI than Ctrl+Sal mice (M = 2.605, SD = 0.216), MD = 0.759, $q = 2.439$, $p = 0.018$. Moreover, MK+Sal (M = 1.677, SD = 0.395) had an SI that was marginally lower than Ctrl+Sal (M = 2.764, SD = 0.375), MD = 1.087, $q = 1.997$, $p = 0.051$. In males, results showed a significant main effect of exposure on SI, $F(1,24) = 9.002$, $p = 0.006$. Post hoc comparisons revealed that MK+Sal mice (M = 1.777, SD = 0.777) had a significantly lower SI than Ctrl+Sal mice (M = 3.115, SD = 1.481), MD = 1.339, $q = 3$, $p = 0.006$. In females, results showed a significant interaction between exposure and treatment, $F(2,24) = 3.777$, $p = 0.037$. Post hoc comparisons revealed that MK+Sal (M = 1.596, SD = 0.244) had a significantly lower SI than Ctrl+Sal (M = 3.046, SD = 1.834), MD = 1.45, $q = 2.451$, $p = 0.022$ (See Figure 2).

FST: At 7 days, results revealed a significant interaction between exposure and treatment, $F(2,54) = 5.952$, $p = 0.005$. Post hoc comparisons revealed that MK+LowHU (M = 119.114, SD = 67.534) was significantly less immobile than MK+Sal (M = 164.754, SD = 36.058), MD = 45.640, $q = 2.563$, $p = 0.025$, and Ctrl+LowHU (M = 181, 238, SD = 18.418), MD = 62.125, $q = 3.488$, $p < 0.001$. In females, results showed a significant interaction between exposure and treatment, $F(2,24) = 7.180$, $p = 0.004$. Post hoc comparisons revealed MK+LowHU (M = 79.794, SD = 75.388) spent significantly less time being immobile than MK+Sal (M = 161.268, SD = 25.60), MD = 81.474, $q = 3.074$, $p = 0.010$. At 17 days, results were significant, $F(5,54) = 3.058$, $p = 0.017$. Post hoc comparisons revealed that compared to Ctrl+Sal (M = 179.729, SD = 34.305), both MK+Sal (M = 218.972, SD = 27.616), MD = 39.243, $q = 2.984$, $p = 0.018$, and MK+HighHU (M = 223.827, SD = 14.747), MD = 44.098, $q = 3.353$, $p = 0.007$, were significantly more immobile. In males, results trended towards a significant main effect of exposure, $F(1,24) = 3.403$, $p = 0.077$. Post hoc comparisons trended towards MK+Sal mice (M = 208.368, SD = 27.631) being more immobile than control mice (M = 186.820, SD = 35.807), MD = 21.548, $q = 1.845$, $p = 0.077$. In females, results trended towards a significant main effect of exposure, $F(1,24) = 2.976$, $p = 0.097$. Post hoc comparisons revealed that MK+Sal (M = 236.797, SD = 4.435) were significantly more immobile than Ctrl+Sal (M = 196.991, SD = 35.908), MD = 39.806, $q = 2.223$, $p = 0.036$, and MK+LowHU (M = 193.755, SD = 40.899), MD = 43.042, $q = 2.404$, $p = 0.045$ (See Figure 3).

NORT: At 7 days, results were significant, $F(5,52) = 4.148$, $p = 0.003$. Post hoc comparisons revealed that MK+Sal (M = -15.35, SD = 22.633) had a significantly lower difference time with the novel object than Ctrl+Sal (M = 15.436, SD = 22.623), MD = 30.786, $q = 2.77$, $p = 0.033$, Ctrl+HighHU (M = 16.712, SD = 21.955), MD = 32.062, $q = 2.885$, $p = 0.024$, MK+LowHU (M = 29.871, SD = 34.836), MD = 45.221, $q = 3.39$, $p = 0.001$, and MK+HighHU (M = 17.847, SD = 24.296), MD = 33.197, $q = 2.907$, $p = 0.023$. In males, results were significant, $F(5,24) = 3.107$, $p = 0.027$. Post hoc comparisons revealed that MK+Sal (M = -24.120, SD = 24.009) had a significantly lower difference time with the novel object than MK+LowHU (M = 40.368, SD = 43.596), MD = 64.488, $q = 3.518$, $p = 0.008$. In females, results were significant, $F(5,21) = 2.942$, $p = 0.036$. Post hoc comparisons revealed that MK+Sal (M = -6.75, SD = 19.592) had significantly lower difference time with the novel object than Ctrl+Sal (M = 32.28, SD = 10.949), MD = 38.86, $q = 2.845$, $p = 0.04$, and Ctrl+HighHU (M = 31.567, SD = 20.43), MD = 38.147, $q = 2.962$, $p = 0.031$. At 17 days, the full analysis was not significant. In females, results were significant, $F(5,23) = 2.781$, $p = 0.042$. Post hoc comparisons revealed that MK+Sal (M = -15.357, SD = 32.087) had significantly lower difference time with the novel object than MK+LowHU (M = 66.435, SD = 42.025), MD = 81.792, $q = 3.202$, $p = 0.016$ (See Figure 4).

ELISA

CB1R : results revealed a significant difference in CB1R concentration, $F(3,36) = 3.405$, $p = 0.028$. Post hoc comparisons revealed that MK+Sal (M = 868.171, SD = 187.744) had significantly lower CB1R

concentration than Ctrl+Sal (M = 1151.893, SD = 344.877), MD = 283.722, $q = 2.531$, $p = 0.042$, and MK+LowHU (M = 1189.108, SD = 290.687), MD = 320.937, $q = 2.864$, $p = 0.019$. MK+Sal CB1R concentration was marginally less than MK+HighHU (M = 1127.731, SD = 111.976), MD = 259.560, $q = 2.316$, $p = 0.068$. In females, results revealed a significant difference in CB1R concentration, $F(3,16) = 5.404$, $p = 0.009$. Post hoc comparisons revealed that MK+Sal (M = 755.483, SD = 75.661) had significantly lower CB1R concentration than Ctrl+Sal (M = 1188.093, SD = 299.908), MD = 432.610, $q = 3.439$, $p = 0.009$, and MK+HighHU (M = 1197.826, SD = 54.206), MD = 442.343, $q = 3.516$, $p = 0.008$. MK+LowHU (M = 1017.288, SD = 244.288) trended towards a greater CB1R concentration than MK+Sal, MD = 261.805, $q = 2.081$, $p = 0.13$ (See Figure 5).

5-HT1AR : Results revealed a significant difference in 5-HT1AR concentration, $F(3,36) = 12.893$, $p < 0.001$. Post hoc comparisons revealed that MK+Sal (M = 86.386, SD = 30.55) had significantly lower CB1R concentration than Ctrl+Sal (M = 150.418, SD = 39.378), MD = 64.032, $q = 4.963$, $p < 0.001$, MK+LowHU (M = 142.419, SD = 24.399), MD = 56.033, $q = 4.343$, $p < 0.001$, and MK+HighHU (M = 158.752, SD = 15.809), MD = 72.366, $q = 5.609$, $p < 0.001$. In females, results revealed a significant difference in 5-HT1AR concentration, $F(3,16) = 158.263$, $p < 0.001$. Post hoc comparisons revealed that MK+Sal (M = 58.308, SD = 2.539) had significantly lower CB1R concentration than Ctrl+Sal (M = 114.917, SD = 10.129), MD = 56.609, $q = 13.560$, $p < 0.001$, MK+LowHU (M = 120.154, SD = 4.379), MD = 61.846, $q = 14.815$, $p < 0.001$, and MK+HighHU (M = 146.577, SD = 6.785), MD = 88.269, $q = 21.144$, $p < 0.001$. In males, results revealed a significant difference in 5-HT1AR concentration, $F(3,16) = 32.858$, $p < 0.001$. Post hoc comparisons revealed that MK+Sal (M = 114.464, SD = 11.073) had significantly lower CB1R concentration than Ctrl+Sal (M = 185.919, SD = 15.347), MD = 71.456, $q = 9.343$, $p < 0.001$, MK+LowHU (M = 164.684, SD = 9.001), MD = 50.220, $q = 6.567$, $p < 0.001$, and MK+HighHU (M = 170.927, SD = 12.072), MD = 56.463, $q = 7.383$, $p < 0.001$ (See Figure 5).

Discussions

MK-801 was effective in inducing schizophrenia-like behaviors consistent with previous literature (Mabunga et al., 2019; Zou et al., 2008; Langen et al., 2012; Nilsson et al., 2007). Indeed, hyperlocomotion (OFT), social deficits (SIT), immobility (FST), and cognitive deficits (NORT) that were shown in this study are consistent effects of NMDAR antagonists as a pharmacological model of schizophrenia (Lee & Zhou, 2019). These behaviors are hypothesized to be related to MK-801 inducing positive-like (i.e., hyperlocomotion), negative-like (i.e., social withdrawal, immobility), and cognitive-related (i.e., memory impairment) symptoms of schizophrenia (Powell & Miyakawa, 2006). These behavioral deficits occur likely because hypofunction of the NMDAR on parvalbumin (PV)-type gamma-amino-butyric acid (GABA)-producing interneurons impairs cortical activity (Balu, 2016). In turn, several regions and pathways in the brain are either hypo- or hyper-activated and result in altered dopamine, serotonin, and glutamate signaling (Balu, 2016). This results in behavioral changes seen in this study.

The effects of MK-801 were dependent on treatment length and sex. Some studies have shown that MK-801 effects may be dose- and approach- dependent in that a high dose (0.5mg/kg), but not a chronic low dose (0.1mg/kg) increases locomotor activity (Eyjolfsson et al., 2006). Indeed, previous literature does show sex-dependent effects of MK-801 (D'Souza, Harlan, and Garcia, 2002). One example of this is the motor-induced differences of MK-801. In females, it is typical of MK-801 to cause increased activity measured as hyperlocomotion (Feinstein & Kritzer, 2013). However, males do not exhibit the same effects. Instead, males show deficits in other aspects of motor-coordination that may need to be measured in other tests (Feinstein & Kritzer, 2013). Therefore, it is without confidence that we could conclude that MK-801 didn't induce the motor-related effects in males as females; only that the OFT was more sensitive in detecting deficits in females. However, unexpectedly our results showed a synergistic-like effect of MK-801 and HU-580 in the OFT, but only in males. Previous literature shows that cannabinoids can affect locomotive activity in the OFT (Kasten, Zhang, & Boehm, 2019), and effects of cannabis in C57BL/6 can be sex-dependent (Peterson et al., 2023). In addition, stress has been shown to have sex-dependent effects in rodents that can increase locomotive activity in males (Jakovcevski, Schachner, & Morellini, 2008; Franceschelli et al., 2014). It is

possible that the synergistic effect observed here is a complex set of sex-dependent effects building upon each other. For example, stress has been shown to alter eCB signalling, increasing the presence of endogenous cannabinoids (eCBs) (Rademacher et al., 2008). However, in the presence of endogenous cannabinoids, compounds such as CBD and CBDA tend to convert into a CB1R inverse agonist (Navarro et al., 2020; An et al., 2020), likely producing an anxiogenic effect (Sink et al., 2010).

As expected, HU-580 attenuated hyperlocomotion, immobility, and cognitive-deficits. Due to limited literature, the effects of HU-580 must be compared to what is known of its successor compounds CBD and CBDA. HU-580 is comparable with similar mechanisms of action with greater potency suggesting possible greater efficacy (Pertwee et al., 2018; Navarro et al., 2020). These results are consistent with previous studies that showed CBD can block the effects of MK-801 (Kruk-Slomka & Biala, 2021). While CBD and CBDA alone do not seem to affect locomotor activity (Calapai et al., 2022), its regulation of NMDAR via CB1R and 5-HT1AR is likely to be one of the mechanisms that reverses hyperlocomotion (Rodríguez-Muñoz et al., 2016; Yuen et al., 2005). Previous research was able to show that NMDAR antagonism can induce immobility in the FST (Langen et al., 2012), and our study corroborates the findings that compounds such as CBD and HU-580 can significantly reduce immobility behavior (Sales et al., 2019; Sales et al., 2020; Dlugosz et al., 2023). Moreover, CBD (Felipe et al., 2013; Osborne et al., 2017; Leweke et al., 2021) and CBDA (Kim et al., 2023) have been shown to improve or rescue cognitive performance in psychiatric and neurological disorders. Regarding behavioral amelioration, conclusions about receptor correlates seem to be complex. CB1R and 5HT1AR are both implicated in positive, negative, and cognitive symptom functioning of schizophrenia as potential therapeutic targets or pathologies (Dickens et al., 2020; Ceccarini et al., 2013; Borgan et al., 2019; Kim, 2021; Kishi, Meltzer, & Iwata, 2013; Ohno, 2011; Švob Štrac, Pivac, & Mück-Šeler, 2016). Therefore, until follow-up studies isolate the therapeutic mechanisms for each behavior in HU-580, further definitive conclusions cannot be made. In our lab, these investigations are under way.

MK-801 down-regulated both the CB1R and 5-HT1AR in the PFC. One such explanation for this is that MK-801 causes an imbalance in excitation and inhibition activity by hypofunction of NMDAR and recruits CB1R regulation to restabilize excitatory-inhibitor (E/I) balance (Sánchez-Blázquez, Rodríguez-Muñoz, & Garzón, 2014). Such dysregulation causes both stress-related responses (Liang, Chen, & Cheng, 2022) and the employment of regulatory receptors (Sánchez-Blázquez, Rodríguez-Muñoz, & Garzón, 2014). Stress induced by MK-801 could be inducing an increase in eCB production and/or release (e.g., 2-AG; Hillard, 2014; Morena et al., 2016). Such increase would agonize CB1Rs, and if done so chronically, should induce receptor sensitization (Hillard, 2014). Additionally, it is possible that MK-801 is down-regulating CB1R via oxidative stress pathways as chronic stress is known to downregulate CB1R (Liang, Chen, & Cheng, 2022; Morena et al., 2016).

HU-580 blocked the downregulation of CB1R by MK-801 exposure. NMDAR and CB1Rs have a complex relationship, and CB1Rs are known to regulate glutamatergic activity (Sánchez-Blázquez, Rodríguez-Muñoz, & Garzón, 2014). Thus, it is likely that CB1R antagonism mitigated some of the increased regulatory mechanisms of NMDAR hypofunction enough so to prevent internalization and down-regulation (Rodríguez-Muñoz et al., 2016). By introducing an antagonist to mitigate downregulation, it is possible that receptor sensitization is kept normal, and effective regulation of dysfunctional NMDAR activity is maintained. Alternatively, it is possible that by antagonizing CB1R, the oxidative stress response of MK-801 and CB1R are being mitigated and preventing down-regulation (Liang, Chen, & Cheng, 2022; Mukhopadhyay et al., 2010). However, there are limited studies on this topic and further research is needed to elucidate the relationship between NMDAR dysfunction, and eCB synthesis, production, and release.

MK-801 induced down regulation of 5-HT1AR that was blocked by HU-580. Findings of increased 5-HT1AR in the PFC of mice corroborates past literature that showed MK-801 increases 5-HT1AR in the rat brain (Wedzony et al., 1997). The pathology of 5-HT1AR in schizophrenia is extremely complex with regionally specific sensitization of 5-HT1AR (Nikolaus, Müller, & Hautzel, 2016; Razakarivony, Newman-Tancredi, & Zimmer, 2021). Our results corroborate with some literature showing decreased 5-HT1AR in the PFC (Nikolaus, Müller, & Hautzel, 2016). However, literature is not consistent, and some studies report increased

5-HT1AR in the PFC (Bantick et al., 2001). Although these results are at odds with what have been reported (Bantick et al., 2001), 5-HT1AR pathological changes may also be subject to compensatory processes that are contingent upon the animal model used, the duration of MK-801 exposure, the model species and strain used, specific experimental procedures and conditions, and the brain structures of interest in question. Moreover, Inconsistent findings of 5-HT1AR sensitization may be a cause for heterogeneity and/or differential manifestation of schizophrenia symptomatology and there may be a difference in 5-HT1AR sub-types. 5-HT1AR heteroreceptors and autoreceptors are sub-types of 5-HT1AR that mediate serotonergic signalling and could be differentially sensitized in schizophrenia (Albert, 2012). Therefore, a procedural approach that will be able to distinguish these subtypes is recommended and is being investigated in our lab. Another possible explanation for 5-HT1AR downregulation by MK-801 is that mice in this study began as healthy adults with an already established serotonin system. Serotonin receptors, especially 5-HT1AR, are involved in the regulation of both NMDARs and the stress response (Biswal et al., 2015; Flügge et al., 1998; Yuen et al., 2005). Similar to the explanation of CB1R downregulation, 5-HT1Rs are likely over activated in an attempt to regulate NMDAR dysfunction and this may subsequently cause internalization (Yuen et al., 2005).

Overall, our results point towards the occurrence of extremely complex crosstalks among the serotonin, eCB, and glutamatergic systems (Haj-Dahmane & Shen, 2011). Although it may be straightforward to discuss each receptor independently, its also possible that there are on-going interactions among these neurotransmitter and receptor systems that MK-801 or HU-580 may be perturbing in a complex manner (Haj-Dahmane & Shen, 2011). However, the dual CB1R-5-HT1AR mechanism of HU-580 posed an enormous challenge to draw conclusions regarding very specific effects on individual mechanisms occurring in an intact brain in vivo. Thus, follow-up studies are needed with pharmacological challenges to the receptor systems (i.e., opposing agonism/antagonism) to investigate precise mechanisms involved in sensitization.

Our study shows preliminary support for both MK-801 as a model of schizophrenia in mice, and that HU-580 would reverse MK-801-induced deficits. These effects appear to be sex- or dose- dependent and further investigation is required to fully elucidate the therapeutic potential of HU-580. Further, we provide support for the role of the CB1R and 5-HT1AR in regulating NMDAR activity in schizophrenia.

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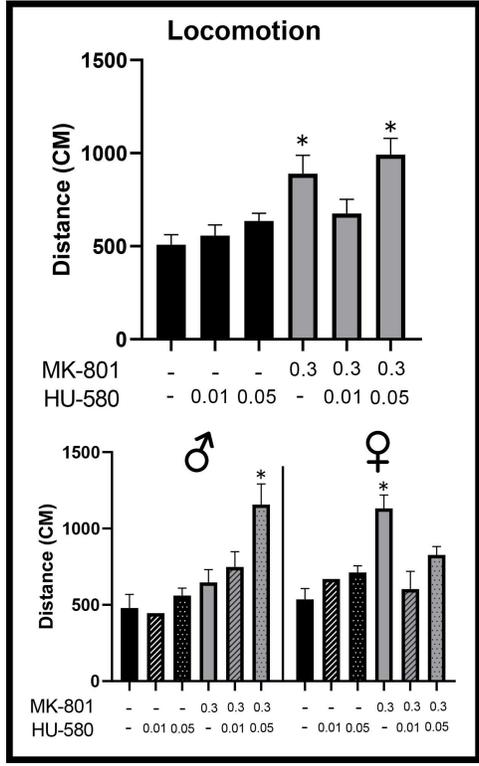


Figure 1. Comparison of the effects of MK-801/HU-580 in the OFT in pooled (left) and sex separated (right) analysis; average (±SEM) distance traveled (CM). Administration of MK-801 (0.3mg/kg, i.p.) daily for 17 days induced hyperlocomotion in sex-dependent manner that was attenuated by low-dose (0.01ug/kg, i.p.) HU-580 treatment. Synergistically, MK-801 and high-dose (0.05ug/kg, i.p.) HU-580 treatment induced hyperlocomotion in male mice.

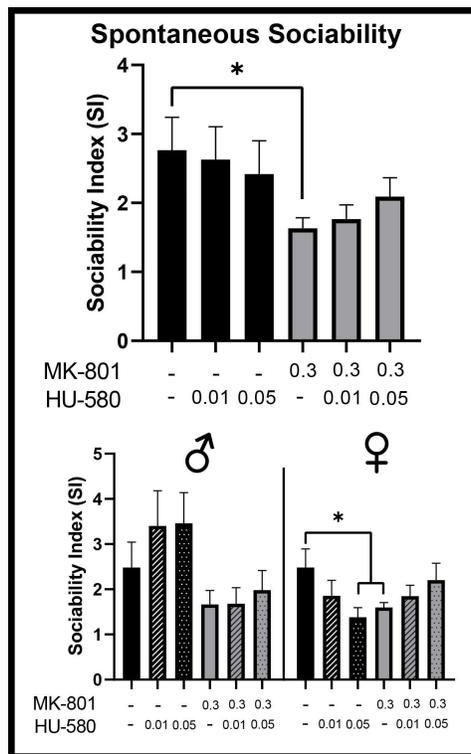


Figure 2. Comparison of the effects of MK-801/HU-580 in the SIT in pooled (left) and sex separated (right) analysis; average (\pm SEM) sociability index (SI). Administration of MK-801 (0.3mg/kg, i.p.) daily for 7 days induced spontaneous sociability deficits (i.e., social withdrawal). High-dose (0.05ug/kg, i.p.) HU-580 treatment induced social withdrawal in female mice at 7 days, but this effect was lost at 17 days.

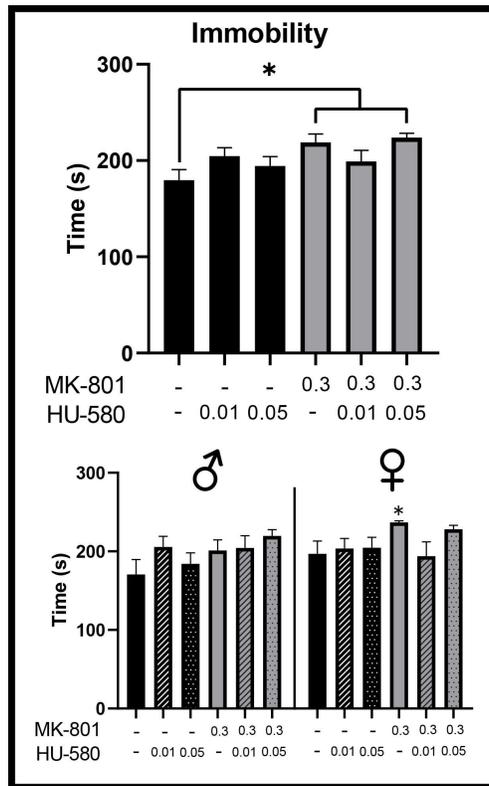


Figure 3. Comparison of the effects of MK-801/HU-580 in the FST in pooled (left) and sex separated (right) analysis; average (\pm SEM) time of immobility (s). Administration of MK-801 (0.3mg/kg, i.p.) daily for 17 days induced immobility (i.e., despair-like behavior) primarily in female mice. Low-dose (0.01ug/kg, i.p.) HU-580 treatment blocked the effects of MK-801 in female mice.

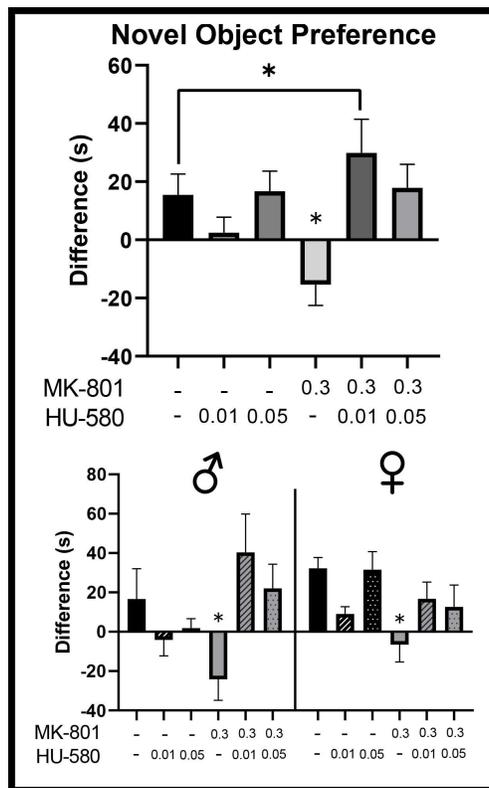


Figure 4. Comparison of the effects of MK-801/HU-580 in the NORT in pooled (left) and sex separated (right) analysis; average (\pm SEM) difference time (s) with the novel object. Administration of MK-801 (0.3mg/kg, i.p.) daily for 7 days induced novel object preference deficits. Both low-dose (0.01ug/kg, i.p.) and high-dose (0.05ug/kg, i.p.) HU-580 treatment blocked the effects of MK-801.

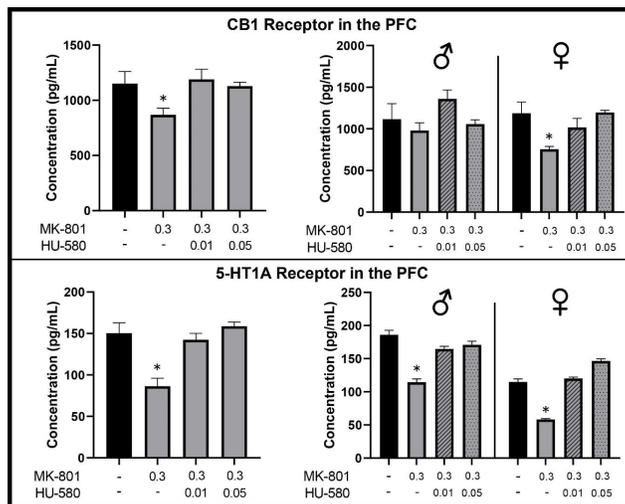


Figure 5. Comparison of the effects of MK-801/HU-580 in the CB1R (top) and 5-HT1AR (bottom) ELISA in pooled (left graphs) and sex separated (right graphs) analysis; average (\pm SEM) concentration (pg/ml). Top: MK-801 (0.3mg/kg, i.p.) daily for 17 days induced down-regulation of the CB1Rs in the PFC of female mice. Both low-dose (0.01ug/kg, i.p.) and high-dose (0.05ug/kg, i.p.) HU-580 treatment blocked the down regulation effects of MK-801. Bottom: MK-801 daily for 17 days induced down regulation of the 5-HT1ARs in the PFC. Both low-dose and high-dose HU-580 treatment blocked the down regulation effects of MK-801.