Published in final edited form as:

J Psychiatr Res. 2020 January ; 120: 103–112. doi:10.1016/j.jpsychires.2019.10.010.

Dual pharmacological inhibitor of endocannabinoid degrading enzymes reduces depressive-like behavior in female rats

Bin Dong^{1,*}, Borehalli M. Shilpa^{1,2,*}, Relish Shah¹, Arjun Goyal¹, Shan Xie¹, Mihran J. Bakalian³, Raymond F. Suckow^{1,3,4}, Thomas B. Cooper^{1,3,4}, J. John Mann^{3,4}, Victoria Arango^{3,4}, K. Yaragudri Vinod^{1,5,6,#}

¹Division of Analytical Psychopharmacology, Nathan Kline Institute for Psychiatric Research, Orangeburg, NY.

²School of Basic and Applied Sciences, Dayananda Sagar University, Bangalore, India.

³New York State Psychiatric Institute, New York, NY.

⁴Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York, NY.

⁵Emotional Brain Institute, Orangeburg, NY.

⁶Department of Child and Adolescent Psychiatry, New York University School of Medicine, New York, NY.

Abstract

Major depressive disorder (MDD) is common, often under-treated and a leading cause of disability and mortality worldwide. The causes of MDD remain unclear, including the role of the endocannabinoid system. Intriguingly, the prevalence of depression is significantly greater in women than men. In this study we examined the role of endocannabinoids in depressive behavior. The levels of endocannabinoids, N-arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG) were measured along with brain derived neurotrophic factor (BDNF) in postmortem ventral striata of female patients with MDD and non-psychiatric controls, and in Wistar Kyoto (WKY) rat, a selectively inbred strain of rat widely used for testing the depressive behavior. The effect of pharmacological elevation of endocannabinoids through inhibition of their catabolizing enzymes (fatty acid amide hydrolase [FAAH] and monoacyl glycerol lipase [MAGL]) on depressive-like phenotype was also assessed in WKY rat. The findings showed lower levels of endocannabinoids and BDNF in the ventral striatum of MDD patients and WKY rats. A dual inhibitor of FAAH and MAGL, JZL195, elevated the endocannabinoids and BDNF levels in ventral striatum, and reduced the depressive-like phenotype in female WKY rats. Collectively, our study

#Address for correspondence: Vinod K. Yaragudri, Ph.D, Nathan Kline Institute for Psychiatric Research, 140 Old Orangeburg Road, Orangeburg, NY 10962, Phone: 845-398-5449, Fax: 845-398-5451, vinod.yaragudri@nki.rfmh.org.

*Equally Contributed to this study.

Conflicts of Interest

There are no conflicts of interest to declare other than Dr. Mann who receives royalties for commercial use of the C-SSRS from the Research Foundation for Mental Hygiene. This article was prepared while Dr. Arango was employed at Columbia University and the New York State Psychiatric Institute. The opinions expressed in this article are the author's own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States government

suggests a blunted endocannabinoid and BDNF signaling in ventral striata of depressive behavior and concludes that endocannabinoid enhancing agents may have an antidepressant effect.

Keywords

Endocannabinoids; JZL195; BDNF; depressive behavior; ventral striatum

1. Introduction

Major depressive disorder (MDD) has a wide range of symptoms and response to treatment, indicating biological heterogeneity (DSM-5; World Health Organization, 2018). Approximately 15% of the world's population has a history of lifetime depression that typically affects more women than men (Bromet et al., 2011; Piccinelli and Wilkinson, 2000). Significant progress has been made in understanding the pathogenesis of depressive illness implicating dysfunctions in multiple signaling pathways (Arango et al., 2002; Owens, 2004; Richelson, 2003). Clinical studies have suggested important roles of the monoaminergic systems in the neurobiology of depression and drugs targeting the serotonergic system are the most widely used antidepressant medications (Arango et al., 2002; Frazer, 1997; Owens, 2004; Piccinelli and Wilkinson, 2000; Richelson, 2003). However, currently available antidepressants are therapeutically inadequate in many patients and new therapeutic targets are needed. Several studies identify BDNF in mediating mood disorders and antidepressant mechanism (Bath et al., 2013; Boldrini et al., 2009; Frazer, 1997; Guilloux et al., 2012; Nemeroff and Vale, 2005; Levinson, 2006; Patki et al., 2013; Wang et al., 2017; Youssef et al., 2018;). Stress can reduce hippocampal BDNF levels, an effect that can be reversed with the use of antidepressants. MDD patients also exhibit low plasma BDNF levels (Lee and Kim, 2010; Phillips, 2017; Polyakova et al., 2015), but since plasma originates from megakaryocytes and platelets it is hard to understand the pathogenic significance of this finding.

The endocannabinoid system that consists of G-protein coupled cannabinoid (CB) receptors, endocannabinoids, and their metabolic enzymes has received much attention recently owing to its role in pathophysiology of many mental disorders. The endocannabinoids are synthesized in the post-synaptic cells and act primarily as retrograde lipid messengers to modulate the functions of excitatory and inhibitory neurotransmission by mainly acting through presynaptic CB1 receptors (Castillo et al., 2012). This neuromodulatory system has been implicated in the regulation of appetite, pain, cognition, mood and emotion (Hillard and Liu, 2014; Jiang et al., 2005; Meyer et al., 2018; Ogawa and Kunugi, 2005; Vinod and Hungund, 2006); however, its role in depressive behavior is not fully understood. A dysfunction of striatal circuitry, especially the nucleus accumbens (ventral striatum), seems to be critically involved in the behavioral deficits associated with reward and motivation (Eisch et al., 2003). This raises a question as to whether the endocannabinoid system in the ventral striatum plays a role in the neurobiology of depressive behavior. Depressive disorder is a complex and multifactorial illness with important genetic and environmental contributing factors. Many animal models have been developed to identify factors underlying depressive behavior and to assess the antidepressant property of drugs. Extensive

behavioral and biochemical studies have characterized that the Wistar Kyoto (WKY) rat is a useful model for understanding the pathophysiology of depressive behavior (Chiba et al., 2010; De La Garza and Mahoney, 2004; Hurley et al., 2013; Paré, 2000; Samuels et al., 2011; Tejani-Butt et al., 1994; Vinod et al., 2012; Will et al., 2003; Yadid et al., 2000).

Despite of higher prevalence of MDD in women than men, the neurobiology of women depression is understudied. Thus, in the present study, we sought to understand the relationship between endocannabinoids and depressive behavior in females. We hypothesized that endocannabinoids levels would be lower in the ventral striata of depressed patients and that pharmacological elevation of endocannabinoids could reduce depressive behavior in animal model. To this end, the endocannabinoids (AEA and 2-AG) and BDNF were analyzed in the postmortem ventral striatal tissues of women with MDD and in female WKY rats. The effect of enhancement of endocannabinoid levels through systemic pharmacological inhibition of FAAH and MAGL enzymes on depressive-like behavior was also assessed in WKY female rats.

2. Materials and methods

2.1 Human Postmortem Brains

2.1.1 Subjects—Brain samples, toxicology, neuropathology examination findings and associated clinical data were made available from the brain tissue collection at the New York State Psychiatric Institute. All tissues were obtained in accordance with protocols approved by the Institutional Review Board. The ventral striata from female subjects with MDD (n=19) and non-psychiatric controls (n=15) were included in this study. All groups were extensively matched for age, postmortem interval (PMI), brain pH and race. Peripheral toxicology was carried out by the Coroner's or Medical Examiner's office in urine, blood or vitreal samples. Brain pH determination and toxicological analyses (over 30 drugs) were performed on cerebellar tissue. Neuropathological examinations conducted by a certified neuropathologist found that all the cases included in this study were free of neuropathology. Psychiatric diagnosis was obtained by structured interviews (SCID I) with family members and/or close friends according to DSM-IV criteria. Psychological autopsies found a lifetime diagnosis of MDD cases and all controls were free of major psychopathology. The demographic, clinical and toxicological information are summarized in Table 1. The brain samples were coded to mask the laboratory staff to the diagnostic groups.

2.1.2 Human Brain dissection—The right hemisphere was sectioned fresh into 10–12 coronal slabs, rapidly frozen in Freon and stored at -80°C . Frozen sections (20 μm) were taken from the coronal slab containing the anterior, precommissural and commissural striatum, and the nucleus accumbens, and then stained for Nissl to aid in the identification of the boundaries of the ventral striatum. Tissue from the ventral striatum was then carefully dissected from the coronal slab and stored at -80°C until assay.

2.1.3 Analysis of AEA and 2-AG—The levels of endocannabinoids, AEA and 2-AG were measured by liquid chromatography-mass spectrometry (LC-MS) using an isotopic dilution procedure (Vinod et al., 2010). Briefly, ventral striatal tissues from human and rats were homogenized in 4 ml of chloroform-methanol-tris buffer (2:1:1, pH 7.4) containing

0.25 mM phenylmethylsulfonyl fluoride (PMSF), 20 μ l of 1% butylated hydroxytoluene (BHT), and 25 ng of AEA-d₈ and 100 ng of 2-AG-d₈ as internal standards. The homogenates were centrifuged at 1,000xg and the organic layer was evaporated to dryness with nitrogen. The residues were reconstituted in ethyl acetate (0.3 ml) and dried down with nitrogen and then redissolved in ethyl alcohol (30 μ l) for LC-MS analysis. The standard curves were fitted with a quadratic equation.

2.1.4 Measurement of BDNF—The BDNF levels were measured using the sandwich enzyme-linked immunosorbent assay (ELISA) Kit (MilliporeSigma, Burlington, MA) in post-synaptic supernatants of human postmortem ventral striatal tissues. The protein content of tissue homogenate was determined by bicinchoninic acid (BCA) assay.

2.2 Animals

The experiments were conducted in adult female Wistar (WIS) and WKY rats (10–16 weeks old; Charles River Laboratory, USA). Rats were housed at 23 $\pm$ 1°C for 12 hr light/dark cycle and given unlimited access to standard chow during the study. All procedures were conducted in accordance with institutional and National Institutes of Health guidelines.

2.3 Drug treatment

The WKY rats were administered once daily with JZL195 (3 mg/kg body weight, i.p.) while control rats (WIS and WKY) received the vehicle (saline containing 4% DMSO, 1% Tween 80 and 1% polyethylene glycol, i.p.) at a volume of 1 ml/kg for 7 consecutive days. This dose was chosen based on the literature search for *in vivo* studies (Long et al., 2009; Leonard et al., 2017). Rats were used for either behavioral tests or they were decapitated 4 and 24 hr after the last injection under deep anesthesia (isoflurane) for biochemical analysis. The study design and timeline of biochemical and behavioral experiments are presented in Fig. 2A

2.4 Behavioral tests

Two sets of rats, one for sucrose preference, and the other for social interaction and forced-swim test were tested as follows. The time points (Fig. 2A) for social interaction and forced swim tests were chosen based on the results of the sucrose preference test. The rat groups were coded to mask the investigators to drug treatment. The experimental designs and resultant data were verified by at least another trained researcher.

2.4.1 Sucrose preference test—Rats were acclimatized to single housing with access to unlimited rat chow and two bottles of water. The effect of systematic administration of JZL195 on sucrose preference was assessed using a two-bottle choice paradigm. Rats were offered access to pre-weighed bottles containing tap water and 1% sucrose and consumption was monitored overnight for 4 consecutive days after the final injection. The WKY rats were pre-exposed to sucrose and water bottles before the drug treatment and were divided into two groups of equal sucrose consumption. Two bottles containing water and 1% sucrose were placed on the cage without rat to control spillage and evaporation. The positions of water and sucrose bottles were alternated every day to avoid place preference. The amount of sucrose intake is expressed as gram of sucrose solution consumed/kg body weight and

sucrose preference as percentage of sucrose consumption over total liquid (sucrose and water) consumption.

2.4.2 Social interaction Test—The social interaction test was conducted 24 hr after the final injection in a solid acrylic box (30 cm × 120 cm × 30 cm) consisting of 3 chambers defined as social, neutral, and anti-social. Each rat was habituated to the arena for 5 min prior to the test. A novel rat (Sprague Dawley) of the same sex was placed in the social chamber before introducing the subject rat. The social behavior of rat (time spent in each chamber and interaction [sniffing and touching]) was monitored for 5 min.

2.4.3 Forced swim test—The effect of JZL195 was assessed in the forced swim test as it is a sensitive and reliable method to assess the antidepressant property of the drugs with high predictive validity (Gobbi et al., 2005; Rittenhouse et al., 2002). After 48 hr following the last injection, rats were tested in the forced swim test. The main behaviors: immobility (no or minimum movement), swimming and climbing were videotaped and assessed during the last 5 min of the 6 min testing session. Rats were considered immobile when floating in water without struggling and making only those movements necessary to keep their heads above the water surface.

2.5 Analysis of plasma and brain JZL195

Rat brain and plasma samples were analysed 4 and 24 hr after the last drug injections. The concentrations of JZL195 in plasma (0.2 ml) and brain homogenate (0.5 ml) were determined by using LC-MS in the positive atmospheric pressure chemical ionization (APCI) mode as described with some modifications (Balla et al., 2018). Following the addition of an internal standard (URB597) to the plasma or brain homogenate, JZL195 and the internal standard were extracted by the addition of 0.5 ml carbonate buffer (pH=9.6) and 3.0 ml of 3% isopropanol in hexane. The samples were mixed gently for 10 min and centrifuged at 2000 rpm. The organic phase was transferred to clean tubes and evaporated to dryness in a vacuum centrifuge. The residue was then reconstituted with 150 µl of methanol:water (1:1), vortexed briefly and transferred to injection vials. The extract was then injected onto an XTerra MS C-18 column (3.0×150mm, 3.5µ, Waters Corp., Milford, MA), eluted with an isocratic mobile phase consisting 0.1% formic acid: methanol: acetonitrile (44:28:28) at a flow rate of 0.6 ml/minute, affording a retention time of 2.7 and 5.6 min for JZL195 and URB597, respectively. Both JZL195 and URB597 were detected at their molecular ions. Each analysis was preceded with an eight-point calibration curve (linear) from 500 to 5 ng/ml. Intra-assay variation (RSD) for JZL195 for all the calibrations standards did not exceed 8.6% (n=8 for each concentration).

2.6 Analysis of AEA, 2-AG and BDNF in rat ventral striatum

Twenty-four hour after the last injections, the ventral striata were isolated on cryostat by micropunch technique. The endocannabinoid assay was conducted using LC-MS method (Vinod et al., 2010) as described above (2.1.3 Analysis of AEA and 2-AG). For BDNF assay, the ventral striatal tissues were homogenized in ice-cold RIPA buffer containing protease and phosphatase inhibitors. The protein content of the tissue homogenate was determined by BCA assay. The BDNF assay was conducted using ELISA Kit.

2.7 Statistical analysis

Analysis of covariance (UNIANOVA), Pearson's correlations and t-tests were performed on human postmortem data using SPSS version 25 (IBM Corp, Armonk, NY). The data obtained from rat studies were analyzed using Prism software (GraphPad 8, San Diego, CA). Differences between the groups were evaluated by either t-test or ANOVA wherever appropriate. All values are presented as Mean $\pm$ SEM and $p < 0.05$ considered to be statistically significant.

3. Results

3.1 Abnormal levels of endocannabinoids and BDNF in ventral striatum of MDD patients

To examine if the central endocannabinoid system is dysregulated in depressive behavior, we measured the levels of two major endocannabinoids; AEA and 2-AG, in postmortem ventral striata of female patients with MDD and non-psychiatric normal controls. Table 1 shows causes of death, toxicology and postmortem brain collection-related indices in the two groups. There were no differences in age ($p = 0.564$; $t = 0.583$, $df = 32$; Control = 15; MDD = 19), PMI ($p = 0.954$; $t = 0.058$, $df = 32$; Control = 15; MDD = 19) and brain pH ($p = 0.721$; $t = 0.343$, $df = 27$; Control = 11; MDD = 18) between the groups (Table 2). The pH data for four control and one MDD subjects were unavailable.

For all analyses we considered PMI, age and pH as potential covariates. Statistically significant covariates were included in ANOVAs. Lower AEA levels were found in MDD ($F_{1,24} = 8.302$; $p = 0.008$; Fig. 1A) with PMI as a significant covariate ($p = 0.032$). 2-AG showed no significant correlates and was lower in MDD compared with controls ($F_{1,23} = 8.684$; $p = 0.007$; Fig. 1B). Lower BDNF levels were observed in MDD ($F_{1,24} = 7.493$; $p = 0.011$; Fig. 1C) with brain pH as a covariate ($p = 0.051$). Post hoc independent-samples t-test found 53% lower levels of AEA in MDD ($t = 2.483$; $df = 18.61$; $p = 0.023$; Fig. 1A), 47% lower 2-AG ($t = 2.77$; $df = 19.04$; $p = 0.012$; Fig. 1B), and 30% lower BDNF ($t = 2.21$; $df = 16.15$; $p = 0.042$; Fig. 1C) compared with controls ($n = 15$). A positive correlation was observed between PMI and AEA levels in the control group ($r = 0.526$, $p = 0.044$) and not MDD group. PMI in the control group but not MDD group was positively correlated with BDNF levels ($r = 0.722$, $p = 0.012$). All other correlations were not statistically significant. Brain toxicology revealed presence of antidepressants in two MDD cases. The statistical analysis without the data from these cases still showed presence of lower levels of AEA ($F_{1,22} = 8.431$, $p = 0.008$), 2-AG ($F_{1,21} = 8.329$, $p = 0.009$), and BDNF ($F_{1,22} = 6.30$, $p = 0.02$) compared with controls.

3.2 JZL195 increases sucrose consumption in female WKY rats

Next, we sought to determine whether elevating endocannabinoids might mitigate depressive behavior as we found low levels of endocannabinoids in MDD. We employed a pharmacological inhibitor of both FAAH and MAGL enzymes, JZL195, to assess an effect on the depressive-like phenotype in WKY rats. The WKY rats consumed significantly less sucrose than WIS rats ($p < 0.0001$; $t = 5.401$; $df = 26$; Fig. 2B) without a difference in sucrose preference ($p = 0.243$; $t = 1.195$; $df = 25$; Fig. 2C) prior to drug administration. The WKY rats received either vehicle or JZL195 treatment and the groups did not differ on sucrose consumption ($p = 0.836$; $t = 0.209$; $df = 17$). Two-way ANOVA indicated an overall effect of

strain [$p<0.0001$; $F_{1,17}=9.40$] and drug administration [$p=0.007$; $F_{3,50}=2.474$] on sucrose consumption without significant interaction between strain and day [$p=0.072$; $F_{3,50}=2.47$] following vehicle treatment (Fig. 2B). A main effect of JZL195 on sucrose consumption was observed in WKY rats compared to vehicle injected WKY rats [$p=0.0003$; $F_{3,42}=0.42$; Fig. 2B]. Post-hoc Bonferroni multiple comparison test showed a modest increase in sucrose consumption by JZL195 on post-injection day 2 [$p=0.041$; $t=2.59$; $df=68$] and 3 [$p=0.045$; $t=2.63$; $df=68$; Fig. 2B]. No significant effects of strain [$p=0.386$; $F_{3,68}=1.027$], day [$p=0.562$; $F_{3,68}=0.688$] and JZL195 treatment [$p=0.437$; $F_{1,68}=0.610$] were observed on sucrose preference during post-injection testing (Fig. 2C). Further within the group analysis revealed a significant increase in sucrose preference only in JZL195 treated WKY rats on days 9 [$p=0.011$] and 10 [$p=0.011$] compared to pre-injection day (Fig. 2C). The social interaction and forced swim tests were conducted based on the results of sucrose preference test that showed a significant effect on sucrose consumption 24 hr after last injection of JZL195.

3.3 JZL195 increases social interaction and reduces behavioral despair in female WKY rats

In the social interaction test, WKY rats administered with JZL195 spent more time in the social chamber compared with vehicle administered WKY rats ($p=0.016$; $t=2.65$; $df=17$; Fig. 3A). However, vehicle administered WKY rats spent more time in the social chamber than vehicle WIS rats ($p=0.0004$; $t=4.22$; $df=20$; Fig. 3A). JZL195 administered WKY rats spent less time in the neutral chamber than control WKY rats ($p=0.029$; $t=2.377$; $df=17$; Fig. 3B). JZL195 and vehicle-administered WKY rats spent similar amounts of time in the anti-social chamber ($p=0.933$; $t=0.085$; $df=19$; Fig. 3C) but WKY vehicle administered rats spent significantly less time in the anti-social chamber than vehicle WIS rats ($p<0.0001$; $t=5.176$; $df=21$; Fig. 3C). The social interaction was lower in vehicle WKY rats compared with vehicle WIS rats ($p=0.031$; $t=1.971$; $df=21$; Fig. 3D) and JZL195 treatment facilitated the social interaction in WKY rats compared with control WKY rats ($p=0.016$; $t=2.61$; $df=19$; Fig. 3D). In the forced swim test, WKY rats showed a markedly more immobility compared with WIS rats ($p<0.0001$; $t=12.04$; $df=20$; Fig. 4). A subtle reduction in immobility in WKY rats was observed by JZL195 administration relative to the control group ($p=0.03$; $t=2.261$; $df=23$; Fig. 4).

3.4 Effect of JZL195 on endocannabinoids and BDNF in rat ventral striatum

To further understand the biochemical effects of JZL195 administration, the endocannabinoids and BDNF levels were measured in ventral striatal tissues 24 hr post-injection. The levels of AEA ($p=0.018$; $t=2.762$; $df=11$; Fig. 5A), 2-AG ($p<0.0001$; $t=6.386$; $df=12$; Fig. 5B) and BDNF ($p=0.037$; $t=1.941$; $df=13$; Fig. 5C) were lower in ventral striata of vehicle treated WKY rats than vehicle treated WIS rats ($n=6-8$ in each group). JZL195 administration increased AEA ($p=0.028$; $t=2.555$; $df=10$; Fig. 5A), 2-AG ($p=0.021$; $t=2.673$; $df=11$; Fig. 5B) and BDNF levels ($p=0.034$; $t=2.347$; $df=14$; Fig. 5C) when compared to vehicle treated WKY rats ($n=6-9$ in each group).

3.5 Pharmacokinetics of JZL195 in female WKY rats

We next measured the concentrations of JZL195 in blood and brain to further examine its bioavailability. The levels of JZL195 reached 46.8 ng/ml in plasma 4 hr post-injection and it significantly decreased to 0.76 ng/ml at 24 hr post-injection when rats were administered 3 mg/kg for 7 days ($p=0.0002$; $t=6.498$; $df=8$; Fig. 6A). The concentration of JZL195 in the brain was 71.3 and 5.7 ng/g at 4 and 24 hr following its administration, respectively ($p=0.0002$; $t=6.551$; $df=8$; Fig. 6B).

4. Discussion

MDD, a chronic and challenging psychiatric illness, is more prevalent in women who exhibit greater symptom severity (Grigoriadis and Robinson, 2007; Kessler et al., 2005; Kornstein et al., 2000). The present study was designed to examine the role of endocannabinoids in depressive behavior in females. We found low levels of two major endocannabinoids, AEA and 2-AG in postmortem ventral striata of females diagnosed with MDD. The ventral striatum has been shown to play a central role in reward and motivation related processes reported to be dysfunctional in mood disorders (Hamilton et al., 2018; Jenkins et al., 2018). Endocannabinoid deficiency in this brain region may impact reward processing and thereby result in anhedonia, a major symptom of clinical depression.

Previous studies report low serum concentrations of AEA and 2-AG in women with major depression (Hill et al., 2008, 2009). Moderate exercise also elevates serum endocannabinoids and that negatively correlates with depressive symptoms (Brellenthin et al., 2017; Meyer et al., 2019). Although the relationship between endocannabinoid levels in serum/plasma and brain is currently unknown, the circulating AEA and 2-AG might have central effects to some degree as these lipids can readily cross the blood brain barrier (Willoughby et al., 1997) and activate reward processes. This is in line with the studies which showed reward-seeking behavior in rats following intravenous administration of endocannabinoids (Justinova et al., 2005, 2011). It remains to be determined if circulating endocannabinoids could serve as biomarkers for the brain endocannabinoids, and for the diagnosis and prognosis of MDD.

Increasing evidence also suggests that BDNF plays a key role in pathophysiology of depression and that antidepressants, in part, exert their effects through regulation of BDNF function. Serum BDNF levels have been found to be lower in depressed patients and are normalized by antidepressant treatment (Gonul et al., 2005). We observed lower postmortem levels of BDNF in the ventral striatum of MDD patients. This is consistent with reported reduction in BDNF gene and protein in the amygdala of female subjects with MDD (Guilloux et al., 2012). Similarly, the blunted BDNF levels in ventral striatum of WKY rats were enhanced by JZL195 treatment. The previous study has also shown an AEA induced increase in brain BDNF in WKY rats through pharmacological inhibition of FAAH (Vinod et al., 2012). An antidepressant effect of cannabidiol (CBD) in animal model through activation of BDNF-TrkB signaling has recently been reported (Sales et al., 2019). CBD could also potentiate this pathway and neuroplasticity through activation of endocannabinoid system (Fogaca et al., 2018). A study by Berghuis et al., (2005) has further shown endocannabinoids could use the TrkB dependent signaling pathways to regulate

neurotrophin signaling in interneurons suggesting an existence of functional interaction between CB1 and TrkB receptor signaling in the brain. Together, these studies provide evidence for the reduced BDNF function in brain regions which are recruited in mediating mood related behaviors, and endocannabinoids seems to potentiate the BDNF-Trk function through a tonic control of BDNF expression by activation of CB1 receptor.

To determine whether elevation of endocannabinoids mitigates depressive behavior, we assessed the behavioral effects of elevation of AEA and 2-AG using a well-established model of depression, the WKY rat (De La Garza and Mahoney, 2004; Hurley et al., 2013; Chiba et al., 2010; Paré, 2000; Samuels et al., 2011; Tejani-Butt et al., 1994; Vinod et al., 2012; Will et al., 2003; Yadid et al., 2000). These rats are unresponsive to many currently available antidepressants, a trait most likely associated with a strong genetic underpinning. Thus, this model appears to be better suited for understanding the genetic basis of depression as well as treatment-resistant depression. We assessed the effect of simultaneous pharmacological inhibition of FAAH and MAGL enzymes by JZL195 on depressive phenotype in female WKY rats. In the forced swim test, WKY rats showed a greater immobility than WIS rats. This behavioral despair was reduced moderately in WKY rats 48 hr post-injection of JZL195.

A reduction in consumption of a palatable sweet solution is behavior indicative of anhedonia-like response (decreased response to rewards) in rodent models of depression (Willner, 2005). In the present study, the WKY rats consumed less sucrose than WIS rats and JZL195 increased sucrose intake in WKY rats, suggesting an increase in reward sensitivity in response to elevation of AEA and 2-AG induced by JZL195. Previous studies suggest existence of dysfunctions of the ventral striatum and dopaminergic system in MDD (Hernandez and Cheer, 2012; Jenkins et al., 2018; Kuter et al., 2011). The palatable food and drugs of abuse have been shown to recruit reward circuitry involving the dopaminergic system in NAc. Blockade of CB1 receptor function reduces sucrose (De Luca et al., 2012) and alcohol intake by suppressing dopamine release in NAc (Rittenhouse et al., 2002). Both AEA and 2-AG, in addition to pharmacological activation of the CB1 receptors, elevate accumbal dopamine release (De Luca et al., 2012, 2014; Solinas et al., 2006) and increase hedonic taste (De Luca et al., 2012). Interestingly, JZL195 increases consumption of highly palatable food (liquid vanilla Ensure) in stressed mice (Bedse et al., 2018). Thus, the behavioral effect of JZL195 observed in our study is most likely associated with endocannabinoid-mediated activation of dopaminergic signaling in ventral striatum that promotes reward and motivation related processes. Whether the modulation of endocannabinoids in rat model of depression leads to synaptic repatterning in ventral striatum need to be determined.

MDD also includes deficits in sociability (social withdrawal) and we investigated whether elevation in endocannabinoids could alter social interaction in WKY rats. Unexpectedly, the WIS rats spent less time in the social chamber where the unfamiliar rat was kept compared to WKY rats. This is mainly due to greater exploratory behavior of WIS rat (Tizabi et al., 2010) but they were more engaged in social interactions (snipping and touching) than WKY rats. JZL195 increased time spent in the social chamber and interaction relative to vehicle treated WKY rats. Interestingly, JZL195-administered WKY rats spent less time in the

neutral chamber than control WKY rats. This suggests that an elevation of endocannabinoids induced by JZL195 administration suppresses social fear-related behavior in WKY rats and that correlates with a prior study that showed enhancement of social interaction by JZL195 treatment in stress-naïve Sprague Dawley rats at a lower dose (Manduca et al., 2015).

A growing body of literature suggests the recruitment of the brain endocannabinoids in fear and anxiety related behaviors (Bedse et al., 2018; Fowler, 2015; Jiang et al., 2005; Manduca et al., 2015). Pretreatment with the MAGL inhibitor (JZL184), reduces affective disturbances in alcohol withdrawn mice (Holleran et al., 2016). A recent study also showed more robust effects of JZL184 than that of PF-3845 (FAAH inhibitor) on stress (foot shock or restraint)-induced fear-related tests in mice (Bedse et al., 2018). In addition, both JZL184 and JZL195 decreased the number of fecal boli, but JZL195 did not show anxiolytic effects on fear-related behavior (Bedse et al., 2018). This may imply that an increase in AEA or 2-AG *per se* reduces fear-related behavior in mouse models but simultaneous elevation of both AEA and 2-AG promotes social interaction in WKY rats. The discrepancies in these results are likely attributed to the use of context dependent behavioral tests, drug dosage, and the sex and strain of the rodents. The effects of JZL195 on fear-related phenotype in WKY rats need to be further evaluated in detail.

The biological actions of AEA and 2-AG appear to be terminated by a two-step process including their reuptake into cells followed by intracellular hydrolysis, although the reuptake mechanism remains unclear. AEA and 2-AG are primarily metabolized by FAAH and MAGL, respectively. JZL195 inhibits both FAAH and MAGL and thereby increases AEA and 2-AG in NAc and other brain regions such as prefrontal cortex without affecting the locomotor activity in WIS rats at low dose (Long et al., 2009; Seillier et al., 2014; Wiskerke et al., 2012). In the current study, JZL195 (at dose 3 mg/kg for 7 days) led to an elevation of AEA and 2-AG in ventral striatum of WKY rats after 24 hr of its administration while our pharmacokinetic study indicated the presence of significantly lower concentrations of JZL195 at 24 hr post-injection in both the brain and plasma of WKY rats. However, an improvement in depressive behavior sustained for at least 2 days post-drug treatment as observed in sucrose preference and forced-swim tests. The exact mechanism of this behavioral effect is unknown, but it could be attributed to non-readily dissociable binding of JZL195 to FAAH and MAGL (Long et al., 2009). The inhibition of these enzymes could continue for a longer time despite elimination of JZL195 from the circulation. Whether metabolite/s of JZL195 also contributed to the behavioral effects need to be studied. The use of multiple dosage and testing intervals could provide clear profile of biochemical and behavioral effects JZL195.

Administration of JZL195 has been shown to elevate both AEA and 2-AG levels in the rodent brains (Belluomo et al., 2015; Long et al., 2009; Manduca et al., 2015; Wiskerke et al., 2012) as seen in the present study. Additionally, attenuation of the JZL195-induced biochemical and behavioral effects were shown to be mediated through CB1 receptor (Long et al., 2009; Manduca et al., 2015). Conversely, there are some apparent antagonism between the production of AEA and 2-AG (Lee et al. 2015; Maccarrone et al. 2008). A further study is needed to delineate if antagonism of production of AEA and 2-AG occurs in brain-region specific, dose and time-dependent manner. Although both CB1 and CB2 receptors have been

shown to mediate endocannabinoid effects, a detailed elucidation of contributions of each of these receptors and AEA effect through vanilloid receptor could further provide underlying mechanism of behavioral effects of JZL195.

Both the MDD and control groups are carefully matched for age, PMI and brain pH. However, potential antemortem confounding effects (such as their mood at the time of death) are possible. Depressive episodes in women may also be partly associated with hormonal fluctuations especially during periods where estrogen levels undergo rapid change (Shors and Leuner, 2012). Estrogen can also recruit the endocannabinoid system to modulate mood and emotion (Gorzalka and Dang, 2012; Hill et al., 2007; Scorticati et al., 2004). The effects of sex hormones on biochemical and behavioral parameters were not teased out in the present study and the influence of sex hormones on the endocannabinoid system and behavioral outcome cannot be ruled out.

Future studies should examine other components of the endocannabinoid system in larger sample sizes, and further assess the effects of JZL195 and other endocannabinoid enhancing agents in additional animal models of depression. In summary, the present study suggests that the hypoactivity of endocannabinoid-mediated signaling in the ventral striatum may be one of the contributing factors to females with MDD. The dual FAAH and MAGL inhibitor JZL195, diminishes the behavioral despair, promotes reward sensitivity and sociability in female WKY rats, suggesting that endocannabinoid enhancing agents have potential as antidepressants.

Acknowledgements

This study is supported by a grant from the National Institutes of Health (MH106935, MH085079 and MH40210). We thank Catia M. Teixeira, Ph.D. for her assistance with the social interaction experiment.

References

1. Arango V, Underwood MD, Mann JJ, 2002 Serotonin brain circuits involved in major depression and suicide. *Prog. Brain Res* 136, 443–453. 10.1016/S0079-6123(02)36037-0 [PubMed: 12143401]
2. Association AP, 2013 Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). American Psychiatric Pub.
3. Balla A, Dong B, Shilpa BM, Vemuri K, Makriyannis A, Pandey SC, Sershen H, Suckow RF, Vinod KY, 2018 Cannabinoid-1 receptor neutral antagonist reduces binge-like alcohol consumption and alcohol-induced accumbal dopaminergic signaling. *Neuropharmacology* 131, 200–208. 10.1016/j.neuropharm.2017.10.040 [PubMed: 29109060]
4. Bath KG, Schilit A, Lee FS, 2013 Stress effects on BDNF expression: effects of age, sex, and form of stress. *Neuroscience* 239, 149–156. 10.1016/j.neuroscience.2013.01.074 [PubMed: 23402850]
5. Bedse G, Bluett RJ, Patrick TA, Romness NK, Gaulden AD, Kingsley PJ, Plath N, Marnett LJ, Patel S, 2018 Therapeutic endocannabinoid augmentation for mood and anxiety disorders: comparative profiling of FAAH, MAGL and dual inhibitors. *Transl Psychiatry* 8 10.1038/s41398-018-0141-7
6. Belluomo I, Matias I, Pernègre C, Marsicano G, Chaouloff F, 2015 Opposite control of frontocortical 2-arachidonoylglycerol turnover rate by cannabinoid type-1 receptors located on glutamatergic neurons and on astrocytes. *J Neurochem* 133, 26–37. <https://doi.org/10.1111/jnc.13044> [PubMed: 25626460]
7. Berghuis P, Dobszay MB, Wang X, Spano S, Ledda F, Sousa KM, Schulte G, Ernfors P, Mackie K., Paratcha G, Hurd YL, Harkany T, 2005 Endocannabinoids regulate interneuron migration and

- morphogenesis by transactivating the TrkB receptor. *Proc Natl Acad Sci U S A* 102, 19115–20. [PubMed: 16357196]
8. Boldrini M, Underwood MD, Hen R, Rosoklija GB, Dwork AJ, John Mann J, Arango V, 2009 Antidepressants increase neural progenitor cells in the human hippocampus. *Neuropsychopharmacology* 34, 2376–2389. 10.1038/npp.2009.75 [PubMed: 19606083]
 9. Brellenthin AG, Crombie KM, Hillard CJ, Koltyn KF, 2017 Endocannabinoid and Mood Responses to Exercise in Adults with Varying Activity Levels. *Med Sci Sports Exerc* 49, 1688–1696. 10.1249/MSS.0000000000001276 [PubMed: 28319590]
 10. Bromet E, Andrade LH, Hwang I, Sampson NA, Alonso J, de Girolamo G, de Graaf R, Demyttenaere K, Hu C, Iwata N, Karam AN, Kaur J, Kostyuchenko S, Lépine J-P, Levinson D, Matschinger H, Mora MEM, Browne MO, Posada-Villa J, Viana MC, Williams DR, Kessler RC, 2011 Cross-national epidemiology of DSM-IV major depressive episode. *BMC Med* 9, 90 10.1186/1741-7015-9-90 [PubMed: 21791035]
 11. Chiba S, Numakawa T, Ninomiya M, Yoon HS, Kunugi H, 2010 Cabergoline, a dopamine receptor agonist, has an antidepressant-like property and enhances brain-derived neurotrophic factor signaling. *Psychopharmacology (Berl)* 211, 291–301. <http://doi:10.1007/s00213-010-1894-8> [PubMed: 20526584]
 12. Castillo PE, Younts TJ, Chávez AE, Hashimoto-dani Y, 2012 Endocannabinoid signaling and synaptic function. *Neuron*. 76, 70–81. <https://doi:10.1016/j.neuron.2012.09.020>. [PubMed: 23040807]
 13. De La Garza R, Mahoney JJ, 2004 A distinct neurochemical profile in WKY rats at baseline and in response to acute stress: implications for animal models of anxiety and depression. *Brain Res* 1021, 209–218. 10.1016/j.brainres.2004.06.052 [PubMed: 15342269]
 14. De Luca MA, Solinas M, Bimpisidis Z, Goldberg SR, Di Chiara G, 2012 Cannabinoid facilitation of behavioral and biochemical hedonic taste responses. *Neuropharmacology* 63, 161–168. 10.1016/j.neuropharm.2011.10.018 [PubMed: 22063718]
 15. De Luca MA, Valentini V, Bimpisidis Z, Cacciapaglia F, Caboni P, Di Chiara G, 2014 Endocannabinoid 2-Arachidonoylglycerol Self-Administration by Sprague-Dawley Rats and Stimulation of in vivo Dopamine Transmission in the Nucleus Accumbens Shell. *Front Psychiatry* 5, 140 10.3389/fpsy.2014.00140 [PubMed: 25368584]
 16. Eisch AJ, Bolaños CA, de Wit J, Simonak RD, Pudiak CM, Barrot M, Verhaagen J, Nestler EJ, 2003 Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. *Biol. Psychiatry* 54,994–1005. 10.1016/j.biopsych.2003.08.003 [PubMed: 14625141]
 17. Fowler CJ, 2015 The potential of inhibitors of endocannabinoid metabolism as anxiolytic and antidepressive drugs-A practical view. *Eur Neuropsychopharmacol* 25, 749–762. 10.1016/j.euroneuro.2015.02.005 [PubMed: 25791296]
 18. Frazer A, 1997 Pharmacology of antidepressants. *J Clin Psychopharmacol* 17 Suppl 1, 2S–18S.
 19. Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano T, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D, 2005 Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proc. Natl. Acad. Sci. U.S.A* 102, 18620–18625. 10.1073/pnas.0509591102 [PubMed: 16352709]
 20. Gonul AS, Akdeniz F, Taneli F, Donat O, Eker C, Vahip S, 2005 Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. *Eur Arch Psychiatry Clin Neurosci* 255, 381–386. 10.1007/s00406-005-0578-6 [PubMed: 15809771]
 21. Gorzalka BB, Dang SS, 2012 Minireview: Endocannabinoids and Gonadal Hormones: Bidirectional Interactions in Physiology and Behavior. *Endocrinol* 153, 1016–1024. 10.1210/en.2011-1643
 22. Grigoriadis S, Robinson GE, 2007 Gender issues in depression. *Ann Clin Psychiatry* 19, 247255 10.1080/10401230701653294
 23. Guilloux J-P, Douillard-Guilloux G, Kota R, Wang X, Gardier AM, Martinowich K, Tseng GC, Lewis DA, Sibille E, 2012 Molecular evidence for BDNF- and GABA-related dysfunctions in the

- amygdala of female subjects with major depression. *Mol. Psychiatry* 17, 1130–1142. 10.1038/mp.2011.113 [PubMed: 21912391]
24. Hamilton JP, Sacchet MD, Hjørnevik T, Chin FT, Shen B, Kämpe R, Park JH, Knutson BD, Williams LM, Borg N, Zaharchuk G, Camacho MC, Mackey S, Heilig M, Drevets WC, Glover GH, Gambhir SS, Gotlib IH, 2018 Striatal dopamine deficits predict reductions in striatal functional connectivity in major depression: a concurrent 11C-raclopride positron emission tomography and functional magnetic resonance imaging investigation. *Transl Psychiatry* 8, 264 10.1038/s41398-018-0316-2 [PubMed: 30504860]
 25. Hernandez G, Cheer JF, 2012 Effect of CB1 receptor blockade on food-reinforced responding and associated nucleus accumbens neuronal activity in rats. *J. Neurosci* 32, 11467–11477. 10.1523/JNEUROSCI.1833-12.2012 [PubMed: 22895729]
 26. Hill MN, Karacabeyli ES, Gorzalka BB, 2007 Estrogen recruits the endocannabinoid system to modulate emotionality. *Psychoneuroendocrinol* 32, 350–357. 10.1016/j.psyneuen.2007.02.003
 27. Hill MN, Miller GE, Carrier EJ, Gorzalka BB, Hillard CJ, 2009 Circulating endocannabinoids and N-acyl ethanolamines are differentially regulated in major depression and following exposure to social stress. *Psychoneuroendocrinology* 34, 1257–1262. 10.1016/j.psyneuen.2009.03.013 [PubMed: 19394765]
 28. Hill MN, Miller GE, Ho W-SV, Gorzalka BB, Hillard CJ, 2008 Serum endocannabinoid content is altered in females with depressive disorders: a preliminary report. *Pharmacopsychiatry* 41, 48–53. 10.1055/s-2007-993211 [PubMed: 18311684]
 29. Hillard CJ, Liu Q, 2014 Endocannabinoid signaling in the etiology and treatment of major depressive illness. *Curr. Pharm. Des* 20, 3795–3811. [PubMed: 24180398]
 30. Ogawa S, Kunugi H, 2015 Inhibitors of Fatty Acid Amide Hydrolase and Monoacylglycerol Lipase: New Targets for Future Antidepressants. *Curr Neuropharmacol* 13, 760–75. [PubMed: 26630956]
 31. Holleran KM, Wilson HH, Fetterly TL, Bluett RJ, Centanni SW, Gilfarb RA, Rocco LER, Patel S, Winder DG, 2016 Ketamine and MAG Lipase Inhibitor-Dependent Reversal of Evolving Depressive-Like Behavior During Forced Abstinence From Alcohol Drinking. *Neuropsychopharmacology* 41, 2062–2071. 10.1038/npp.2016.3 [PubMed: 26751284]
 32. Hurley LL, Akinfiresoye L, Nwulia E, Kamiya A, Kulkarni AA, Tizabi Y, 2013 Antidepressant-like effects of curcumin in WKY rat model of depression is associated with an increase in hippocampal BDNF. *Behavioural Brain Research* 239, 27–30. 10.1016/j.bbr.2012.10.049 [PubMed: 23142609]
 33. Jenkins LM, Skerrett KA, DelDonno SR, Patron VG, Meyers KK, Peltier S, Zubieta J-K, Langenecker SA, Starkman MN, 2018 Individuals with more severe depression fail to sustain nucleus accumbens activity to preferred music over time. *Psychiatry Res Neuroimaging* 275, 21–27. 10.1016/j.pscychresns.2018.03.002 [PubMed: 29555382]
 34. Jiang W, Zhang Y, Xiao L, Van Cleemput J, Ji S-P, Bai G, Zhang X, 2005 Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. *J. Clin. Invest* 115, 3104–3116. 10.1172/JCI25509 [PubMed: 16224541]
 35. Justinova Z, Solinas M, Tanda G, Redhi GH, Goldberg SR, 2005 The endogenous cannabinoid anandamide and its synthetic analog R(+)-methanandamide are intravenously self-administered by squirrel monkeys. *J. Neurosci* 25, 5645–5650. 10.1523/JNEUROSCI.0951-05.2005 [PubMed: 15944392]
 36. Justinová Z, Yasar S, Redhi GH, Goldberg SR, 2011 The endogenous cannabinoid 2-arachidonoylglycerol is intravenously self-administered by squirrel monkeys. *J. Neurosci* 31, 7043–7048. 10.1523/JNEUROSCI.6058-10.2011 [PubMed: 21562266]
 37. Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE, 2005 Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry* 62, 617–627. 10.1001/archpsyc.62.6.617 [PubMed: 15939839]
 38. Kornstein SG, Schatzberg AF, Thase ME, Yonkers KA, McCullough JP, Keitner GI, Gelenberg AJ, Ryan CE, Hess AL, Harrison W, Davis SM, Keller MB, 2000 Gender differences in chronic major and double depression. *J Affect Disord* 60, 1–11. [PubMed: 10940442]

39. Kuter K, Kolasiewicz W, Gotembiowska K, Dziubina A, Schulze G, Berghauzen K, Wardas J, Ossowska K, 2011 Partial lesion of the dopaminergic innervation of the ventral striatum induces “depressive-like” behavior of rats. *Pharmacol Rep* 63, 1383–1392. [PubMed: 22358086]
40. Lazzarin N, Valensise H, Bari M, Ubaldi F, Battista N, Finazzi-Agrò A, Maccarrone M, 2004 Fluctuations of fatty acid amide hydrolase and anandamide levels during the human ovulatory cycle. *Gynecological Endocrinology* 18, 212–218. 10.1080/09513590410001692492 [PubMed: 15293893]
41. Lee BH, Kim YK, 2010 The Roles of BDNF in the Pathophysiology of Major Depression and in Antidepressant Treatment. *Psychiatry Investigation* 7, 231–234. 10.4306/pi.2010.7.4.231 [PubMed: 21253405]
42. Lee SH, Ledri M, Tóth B, Marchionni I, Henstridge CM, Dudok B, Kenesei K, Barna L, Szabó SI, Renkecz T, Oberoi M, Watanabe M, Limoli CL, Horvai G, Soltesz I, Katona I, 2015 Multiple Forms of Endocannabinoid and Endovanilloid Signaling Regulate the Tonic Control of GABA Release. *J Neurosci* 35:10039–57 <http://doi:10.1523/JNEUROSCI.4112-14.2015> [PubMed: 26157003]
43. Levinson DF, 2006 The genetics of depression: a review. *Biol. Psychiatry* 60, 84–92. 10.1016/j.biopsych.2005.08.024 [PubMed: 16300747]
44. Long JZ, Nomura DK, Vann RE, Walentiny DM, Booker L, Jin X, Burston JJ, Sim-Selley LJ, Lichtman AH, Wiley JL, Cravatt BF, 2009 Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. *Proc. Natl. Acad. Sci. U.S.A* 106, 20270–20275. 10.1073/pnas.0909411106 [PubMed: 19918051]
45. Leonard MZ, Alapafuja SO, Ji L, Shukla VG, Liu Y, Nikas SP, Makriyannis A, Bergman J, Kangas BD, 2017 Cannabinoid CB(1) Discrimination: Effects of Endocannabinoids and Catabolic Enzyme Inhibitors. *J Pharmacol Exp Ther* 363, 314–323. doi: 10.1124/jpet.117.244392. [PubMed: 28947487]
46. Maccarrone M, Rossi S, Bari M, De Chiara V, Fezza F, Musella A, Gasperi V, Prosperetti C, Bernardi G, Finazzi-Agrò A, Cravatt BF, Centonze D, 2008 Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum. *Nat Neurosci* 11, 152–9. <http://doi:10.1038/nn2042>. [PubMed: 18204441]
47. Manduca A, Morena M, Campolongo P, Servadio M, Palmery M, Trabace L, Hill MN, Vanderschuren LJMJ, Cuomo V, Trezza V, 2015 Distinct roles of the endocannabinoids anandamide and 2-arachidonoylglycerol in social behavior and emotionality at different developmental ages in rats. *Eur Neuropsychopharmacol* 25, 1362–1374. 10.1016/j.euroneuro.2015.04.005 [PubMed: 25914159]
48. Meyer JD, Crombie KM, Cook DB, Hillard CJ, Koltyn KF, 2019 Serum Endocannabinoid and Mood Changes after Exercise in Major Depressive Disorder. *Med Sci Sports Exerc* 10.1249/MSS.0000000000002006
49. Meyer HC, Lee FS, Gee DG, 2018 The Role of the Endocannabinoid System and Genetic Variation in Adolescent Brain Development. *Neuropsychopharmacol* 43, 21–33. 10.1038/npp.2017.143
50. Nemeroff CB, Vale WW, 2005 The neurobiology of depression: inroads to treatment and new drug discovery. *J Clin Psychiatry* 66 Suppl 7, 5–13.
51. Owens MJ, 2004 Selectivity of antidepressants: from the monoamine hypothesis of depression to the SSRI revolution and beyond. *J Clin Psychiatry* 65 Suppl 4, 5–10.
52. Paré WP, 2000 Investigatory behavior of a novel conspecific by Wistar Kyoto, Wistar and Sprague-Dawley rats. *Brain Res. Bull* 53, 759–765. 10.1016/S0361-9230(00)00362-2 [PubMed: 11179840]
53. Patki G, Solanki N, Atrooz F, Allam F, Salim S, 2013 Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. *Brain Res* 1539, 73–86. 10.1016/j.brainres.2013.09.033 [PubMed: 24096214]
54. Phillips C, 2017 Brain-Derived Neurotrophic Factor, Depression, and Physical Activity: Making the Neuroplastic Connection. *Neural Plast* 2017, 7260130 10.1155/2017/7260130 [PubMed: 28928987]
55. Piccinelli M, Wilkinson G, 2000 Gender differences in depression. Critical review. *Br J Psychiatry* 177, 486–492. 10.1192/bjp.177.6.486 [PubMed: 11102321]

56. Polyakova M, Stuke K, Schuemberg K, Mueller K, Schoenknecht P, Schroeter ML, 2015 BDNF as a biomarker for successful treatment of mood disorders: a systematic & quantitative metaanalysis. *J Affect Disord* 174, 432–440. 10.1016/j.jad.2014.11.044 [PubMed: 25553404]
57. Richelson E, 2003 Interactions of antidepressants with neurotransmitter transporters and receptors and their clinical relevance. *J Clin Psychiatry* 64 Suppl 13, 5–12.
58. Rittenhouse PA, López-Rubalcava C, Stanwood GD, Lucki I, 2002 Amplified behavioral and endocrine responses to forced swim stress in the Wistar-Kyoto rat. *Psychoneuroendocrinology* 27, 303–318. 10.1016/S0306-4530(01)00052-X [PubMed: 11818168]
59. Samuels BA, Leonardo ED, Gadiant R, Williams A, Zhou J, David DJ, Gardier AM, Wong EHF, Hen R, 2011 Modeling treatment-resistant depression. *Neuropharmacology* 61, 408–413. 10.1016/j.neuropharm.2011.02.017 [PubMed: 21356220]
60. Sales AJ, Fogaca MV, Sartim AG, Pereira VS, Wegener G, Guimaraes FS, Joca SRL, 2019 Cannabidiol Induces Rapid and Sustained Antidepressant-Like Effects Through Increased BDNF Signaling and Synaptogenesis in the Prefrontal Cortex. *Mol. Neurobiol* 56, 1070–1081. 10.1007/s12035-018-1143-4 [PubMed: 29869197]
61. Fogaca MV, Campos AC, Coelho LD, Duman RS, Guimarães FS, 2018 The anxiolytic effects of cannabidiol in chronically stressed mice are mediated by the endocannabinoid system: role of neurogenesis and dendritic remodeling. *Neuropharmacology* 3, 22–33. <https://doi.org/10.1016/j.neuropharm.2018.03.001>
62. Schmid PC, Paria BC, Krebsbach RJ, Schmid HHO, Dey SK, 1997 Changes in anandamide levels in mouse uterus are associated with uterine receptivity for embryo implantation. *Proceedings of the National Academy of Sciences* 94, 4188–4192. 10.1073/pnas.94.8.4188
63. Scorticati C, Fernandez-Solari J, De Laurentiis A, Mohn C, Prestifilippo JP, Lasaga M, Seilicovich A, Billi S, Franchi A, McCann SM, Rettori V, 2004 The inhibitory effect of anandamide on luteinizing hormone-releasing hormone secretion is reversed by estrogen. *Proceedings of the National Academy of Sciences* 101, 11891–11896. 10.1073/pnas.0404366101
64. Seillier A, Dominguez Aguilar D, Giuffrida A, 2014 The dual FAAH/MAGL inhibitor JZL195 has enhanced effects on endocannabinoid transmission and motor behavior in rats as compared to those of the MAGL inhibitor JZL184. *Pharmacol. Biochem. Behav* 124, 153–159. 10.1016/j.pbb.2014.05.022 [PubMed: 24911644]
65. Solinas M, Justinova Z, Goldberg SR, Tanda G, 2006 Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. *J. Neurochem* 98, 408–419. 10.1111/j.1471-4159.2006.03880.x [PubMed: 16805835]
66. Tejani-Butt SM, Paré WP, Yang J, 1994 Effect of repeated novel stressors on depressive behavior and brain norepinephrine receptor system in Sprague-Dawley and Wistar Kyoto (WKY) rats. *Brain Res* 649, 27–35. 10.1016/0006-8993(94)91045-6 [PubMed: 7953642]
67. Tizabi Y, Hauser SR, Tyler KY, Getachew B, Madani R, Sharma Y, Manaye KF, 2010 Effects of nicotine on depressive-like behavior and hippocampal volume of female WKY rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 62–69. 10.1016/j.pnpbp.2009.09.024 [PubMed: 19800382]
68. Vinod KY, Hungund BL, 2006 Role of the endocannabinoid system in depression and suicide. *Trends Pharmacol. Sci* 27, 539–545. 10.1016/j.tips.2006.08.006 [PubMed: 16919786]
69. Vinod KY, Kassir SA, Hungund BL, Cooper TB, Mann JJ, Arango V, 2010 Selective alterations of the CB1 receptors and the fatty acid amide hydrolase in the ventral striatum of alcoholics and suicides. *J Psychiatr Res* 44, 591–597. 10.1016/j.jpsychires.2009.11.013 [PubMed: 20015515]
70. Vinod KY, Xie S, Psychoyos D, Hungund BL, Cooper TB, Tejani-Butt SM, 2012 Dysfunction in Fatty Acid Amide Hydrolase Is Associated with Depressive-Like Behavior in Wistar Kyoto Rats. *PLoS One* 7 10.1371/journal.pone.0036743
71. Wang Q, Timberlake MA, Prall K, Dwivedi Y, 2017 The recent progress in animal models of depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 77, 99–109. 10.1016/j.pnpbp.2017.04.008 [PubMed: 28396255]
72. WHO | [WWW Document], n.d. WHO. URL <https://www.who.int/healthinfo/bodestimates/en/> (accessed 4.30.19).

73. Will CC, Aird F, Redei EE, 2003 Selectively bred Wistar-Kyoto rats: an animal model of depression and hyper-responsiveness to antidepressants. *Mol. Psychiatry* 8, 925–932. 10.1038/sj.mp.4001345 [PubMed: 14593430]
74. Willner P, 2005 Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology* 52, 90–110. 10.1159/000087097 [PubMed: 16037678]
75. Willoughby KA, Moore SF, Martin BR, Ellis EF, 1997 The biodisposition and metabolism of anandamide in mice. *J. Pharmacol. Exp. Ther* 282, 243–247. [PubMed: 9223560]
76. Wiskerke J, Irimia C, Cravatt BF, De Vries TJ, Schoffelmeer ANM, Pattij T, Parsons LH, 2012 Characterization of the effects of reuptake and hydrolysis inhibition on interstitial endocannabinoid levels in the brain: an in vivo microdialysis study. *ACS Chem Neurosci* 3, 407–417. 10.1021/cn300036b [PubMed: 22860210]
77. Yadid G, Nakash R, Deri I, Tamar G, Kinor N, Gispan I, Zangen A, 2000 Elucidation of the neurobiology of depression: insights from a novel genetic animal model. *Prog. Neurobiol* 62, 353–378. 10.1016/S0301-0082(00)00018-6 [PubMed: 10856609]
78. Youssef MM, Underwood MD, Huang Y-Y, Hsiung S-C, Liu Y, Simpson NR, Bakalian MJ, Rosoklija GB, Dwork AJ, Arango V, Mann JJ, 2018 Association of BDNF Val66Met Polymorphism and Brain BDNF Levels with Major Depression and Suicide. *Int. J. Neuropsychopharmacol* 21, 528–538. 10.1093/ijnp/pyy008 [PubMed: 29432620]
79. Shors TJ, Leuner B, 2003 Estrogen-mediated effects on depression and memory formation in females. *J. Aff. Dis* 74, 85–96. [https://doi.org/10.1016/S0165-0327\(02\)00428-7](https://doi.org/10.1016/S0165-0327(02)00428-7).

Fig. 1.

The levels of endocannabinoids, AEA (* $p=0.023$; $n=19$; A) and 2-AG (* $p=0.012$; $n=18$; B), and BDNF (* $p=0.042$; $n=19$; C) were found significantly lower in ventral striata of MDD patients compared to non-psychiatric controls ($n=15$).

(A)

(B)

(C)

Fig. 2.

A timeline of the biochemical and behavioral experiments is depicted in panel (A). Rats were administered once daily with either JZL195 (3 mg/kg, i.p.) or vehicle for 7 consecutive days. The rats were pre-tested for sucrose preference (day 0) and then retested them for 4 consecutive days (7 to 10) after the final injection. A second group of rats were tested for social interaction and forced swim 24 and 48 hr post-injections, respectively. The brains and trunk blood were collected 4 and 24 hr post-injections from a separate group rats for biochemical analysis (endocannabinoids, BDNF, JZL195). In sucrose preference test, WKY rats consumed significantly less sucrose than WIS rats on pre-drug injection (*** $p < 0.0001$; B) without significant effect on sucrose preference ($p = 0.243$; WIS=9; WKY=19; C). An overall effect of strain ($p < 0.0001$) and post-injection day ($p = 0.007$) was observed on sucrose consumption in vehicle injected rats ($n = 9-10$ in each group; B). There was a main effect of

JZL195 administration on sucrose consumption in WKY rats compared to vehicle injected WKY rats ($***p=0.0003$; B) without significant effect on day ($p=0.195$; $n=9-10$ in each group; B). A modest increase in sucrose consumption was observed following JZL195 treatment on day 8 ($*p=0.041$) and 9 ($*p=0.045$). No significant effects of either strain ($p=0.386$), day ($p=0.562$) or JZL195 treatment ($p=0.437$) was observed on sucrose preference during post-injection. However, sucrose preference was increased only in JZL195 treated group on days 9 [$*p=0.011$] and 10 [$*p=0.011$] compared to preinjection day in WKY rats ($n=9-10$ in each group; C).

Fig. 3.

In social interaction test, JZL195 (3 mg/kg, i.p. for 7 days) administered WKY rats spent more time in social chamber compared to control WKY rats (* $p=0.016$) while vehicle treated WKY rats spent more time in social chamber than vehicle treated WIS rats (*** $p=0.0004$; $n=9-13$ in each group; A). JZL195 treated WKY rats spent less time in neutral chamber than control WKY rats (* $p=0.029$; B). JZL195 did not change the time spent in anti-social chamber in WKY rats ($p=0.933$; C) but WKY vehicle treated rat spent less time in anti-social chamber than vehicle injected WIS rats (*** $p<0.0001$; C). A decrease in social interaction was observed in vehicle treated WKY rats than control WIS rats (* $p=0.031$; D) while JZL195 treatment facilitated the social interaction in WKY rats compared to control WKY rats (* $p=0.016$; D).

Fig. 4.

In forced-swim test, WKY rats showed a marked immobility compared to WIS rats (** $p < 0.0001$; $n = 10-13$ in each group). JZL195 (3 mg/kg, i.p. for 7 days) administration led to a modest reduction in total time spent in immobility in WKY rats compared to control WKY group (* $p = 0.03$; $n = 10-11$ in each group).

Fig. 5.

The levels of AEA (* $p=0.018$; A), 2-AG (** $p<0.0001$; B) and BDNF (* $p=0.037$; C) were lower in ventral striata of vehicle treated WKY rats compared to vehicle treated WIS rats ($n=6-8$ in each group). JZL195 (3 mg/kg, i.p. for 7 days) administration increased AEA (* $p=0.028$; A), 2-AG (* $p=0.021$; B) and BDNF levels (* $p=0.034$; C) when compared to vehicle treated WKY rats ($n=6-9$ in each group).

Fig. 6.

The levels of JZL195 in plasma (A) and brain (B) of WKY rats collected 4 and 24 hr post-injection of JZL195 (3 mg/kg, i.p. for 7 days). JZL195 levels were significantly reduced at 24 hr in both plasma (** $p=0.0002$; $n=5$ in each group; A) and brain (** $p=0.0002$; $n=5$ in each group; B) compared to 4 hr post-injection time point.

Table 1.

Demographic and Clinical Characteristics of MDD and Control groups

No.	Group	Age	PMI	Brain pH	Cause of death	Toxicology
01	Control	27	15.0	6.72	MVA (Pedestrian)	None
02	Control	29	16.5	6.29	MVA (Passenger)	None
03	Control	31	12.5	6.66	Natural (Cardiovascular)	Lidocaine
04	Control	35	15.0	6.30	Homicide (Stabbing)	CO
05	Control	40	15.5	5.92	Natural (Cardiovascular)	DU
06	Control	48	05.0	6.33	Natural (Hepatic)	Nicotine/Cotinine
07	Control	51	12.3	6.23	Natural (Cardiovascular)	Lidocaine, Caffeine
08	Control	54	12.0	6.48	Natural (Cardiovascular)	Caffeine
09	Control	54	17.0	NA	Natural (Cardiovascular)	None
10	Control	65	21.0	6.80	MVA (Passenger)	None
11	Control	67	15.0	NA	Homicide (Gunshot)	None
12	Control	71	12.5	NA	Natural (Cardiovascular)	None
13	Control	74	12.0	5.70	Natural (Cardiovascular)	Lidocaine, Caffeine
14	Control	75	07.5	6.33	MVA (Pedestrian)	None
15	Control	75	12.5	NA	Natural (Respiratory)	Lidocaine
01	MDD	24	14.0	6.42	Suicide	None
02	MDD	28	19.0	6.32	Suicide	None
03	MDD	29	04.0	6.36	Suicide	None
04	MDD	31	07.5	6.47	Suicide	ADEP
05	MDD	34	04.0	6.93	Suicide	None
06	MDD	40	18.0	7.03	Suicide	None
07	MDD	41	13.5	6.64	Suicide	CO
08	MDD	42	16.0	6.60	Suicide	None
09	MDD	43	12.0	6.74	Suicide	ACE, ADEP, FLU
10	MDD	50	14.5	6.84	Suicide	None
11	MDD	50	24.0	5.90	Suicide	None
12	MDD	58	09.0	5.71	Suicide	Caffeine
13	MDD	60	06.0	5.50	Suicide	DU
14	MDD	61	11.0	6.83	Suicide	None
15	MDD	63	15.0	6.24	Suicide	DU
16	MDD	69	07.0	6.30	Suicide	Clear
17	MDD	71	19.0	6.40	Suicide	DU
18	MDD	74	24.0	5.90	Suicide	Lidocaine
19	MDD	75	15.5	NA	Suicide	Lidocaine

PMI: Postmortem interval; MDD: Major Depressive Disorder; MVA: Motor Vehicle Accident; GSW: Gun Shot Wound; CO: Carbon Monoxide; ACE: Acetaminophen; ADEP: Antidepressants (Imipramine, Prozac, Paroxetine, Nortryptiline); FLU: Fluoxetine; DU: Data Unavailable

Table 2.

Age, PMI and Brain pH in MDD and Control groups

Group	Age (year)	PMI (hr)	Brain pH
Control	53.1±4.5	13.4±0.98	6.3±0.1
MDD	49.6±3.8	13.3±1.4	6.4±0.1
p-value	p=0.564	p=0.954	p=0.721

No significant differences were observed in age, PMI (postmortem interval) and brain pH between the MDD and control groups.