The Effects of Early Life Stress and Cannabidiol on Behavior and Corticosterone Levels in Male and Female Rats

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ABSTRACT

Organisms must maintain homeostasis that is regulated internally and externally. Fluctuation in homeostasis causes stress. Corticosterone, the main stress hormone in rodents, increases following exposure to acute stressors, such as being placed in a restrainer. However, early developmental experiences including maternal separation shape the development of the stress response into adulthood and impact the expression of depressive and anxiety-like behaviors. Many factors can shape the body's response to stress, for example, a rodent's previous exposure to a past stressor can affect their present behavior. Several studies have shown that female rats have lower or no changes in corticosterone levels compared to the male rats following developmental stress of maternal separation, though most indicate no sex difference. This thesis will explore how early life stress affects rodents' development and corticosterone levels as well as the use of CBD as a potential therapeutic.

The current investigation found no sex differences with prolonged maternal deprivation on either PND3 or PND11. The second article examined the mechanism by which CBD and chronic stressors affects male Wistar rats; subjects displayed increased weight gain as well as hair CORT levels. Studies using maternal separation differ in both the duration of separating pups from their mothers, as well as how many days the daily procedure is continued. Therefore, results are difficult to generalize due to methodological differences. Some evidence suggests cannabidiol may counteract the effects of early life stress. However, more studies should be done to investigate the effects of chronic stress, CBD, and corticosterone levels. Additionally, there would be benefits of more research that looks at sex differences, as most studies were conducted on male rats. This variable makes it harder to generalize the results of how certain stressors and CBD would affect females.

OVERVIEW

In every living creature, there must be a balance. When a creature recognizes something as a stressor or threat, that causes an imbalance which can lead to behavioral and internal changes that help the creature deal with the stressor or threat. These internal changes can include an increase in blood pressure, heart rate, enlargement of the pupils, and release of stress hormones such as corticosterone. These responses are due, in

part, to the activation of the fight or flight system which is controlled by the hypothalamic pituitary adrenal axis (HPA). Corticosterone (CORT) is the main stress hormone in rodents that controls energy regulation and the response to stressors. Prolonged exposure to CORT in the body can lead to adverse effects. However, there are many things that can shape the way the body responds to stress such as previous exposure to stress throughout development.

To study the effects of early life stress (ELS) on development, scientists have done multiple studies exposing rodents to short-term and long-term stress while measuring the effects on CORT levels. This is done by a variety of methods such as being placed in restraints, or by being exposed to a shock, cold temperatures, or maternal separation. These studies have shown that ELS impacts brain development, such as an increase in anxiety and depressive behaviors, and a decrease in learning and memory.

With maternal separation as the stressor, there have been mixed results. Some studies suggest that there are sex differences when it comes to corticosterone levels. Males tend to have higher levels of corticosterone following maternal separation, whereas no differences are often found among females. However, only a few studies have used female rats and the studies using maternal separation differ in both the duration of separation and the daily procedure. Therefore, it is hard to generalize results that come from these studies due to many methodological differences.

Another focus of this investigation was to characterize the potential therapeutic benefit of cannabidiol (CBD) to counteract the effects of ELS. CBD is generally well tolerated and has not been found to have any intoxicating effects. Some studies have shown that CBD administration is effective at treating the behavioral effects of ELS, with the opposite effects on CORT levels. However, only some studies have begun to investigate the role of CBD at preventing psychiatric issues by administering CBD during adolescent development. Some evidence suggests cannabidiol (CBD) can be used to counteract the effects of ELS. CBD is mainly used because it does not cause any toxication effects. It has been shown in a study using female mice that influences memory and impairment but improves behavior. Chronic use of CBD was shown to have, anxiolytic behaviors (García-Gutiérrez et al., 2020).

More studies are needed to look at how ELS influences rodents on both male and female rodents. Since most studies are done on male rodents, more research is also needed to look at how ELS affects females. No research was done on maternally separated rats and how CBD affects the rats. Therefore, research needs to expand and have consistent protocols to determine whether the results are accurate or not.

I. INTRODUCTION

Imagine a rat running through a forest when it hears a fox howl. Adrenaline rushes through their body followed by an influx of stress hormones and adaptive changes such as an increased heart rate, blood pressure, and an enlargement of the pupils. This example demonstrates how our stress response is important for the protection and survival of an organism. Indeed, every living organism must maintain homeostasis. Following a stressor or threat, the imbalance causes stress, leading to behavioral and internal changes that help the organism deal with the stressor or threat (Chrousos, 1992). Therefore, stress can be described as physiological and behavioral changes due to imbalances of homeostasis (Geiker et al., 2018). These responses are due, in part, to the activation of the fight or flight system which is controlled by the hypothalamic-pituitary-adrenal axis (HPA).

As the rat hears the howl, the sympathetic nervous system is activated, triggering the fight or flight response. In this example, the rat's response includes the release of the hormone corticosterone (CORT). CORT is the main stress hormone found in rodents (Joëls et al., 2018) that is released from the adrenal cortex (Mohn et al., 2005). Corticosterone plays a crucial role in regulating energy through stored up glucose and ATP as well as stress response (De Souza et al., 1982; Díaz-Muñoz et al., 2000). For example, corticosterone levels increase following exposure to acute stressors (Consoli et al., 2005) such as a forced swim test, being placed in restraints, shock, temperature fluctuation, limited resources, or maternal separation. However, with exposure to stress, an individual's reaction may vary. Many things can shape the body's response to stress, an example can be seen with a rodent's previous exposure to a past stressor.

The hormonal mechanism from the hypothalamic-pituitary-adrenal axis (HPA) is a longer-lasting response (Godoy et al., 2018). Neuroendocrine hormones released from HPA are involved in regulating equilibrium and responding to stress (Chrousos, 2019). In events of physiological or psychological stress, the

HPA axis is activated which secretes the corticotropin-releasing hormone (CRH) from the hypothalamus. This then triggers the release of the adrenocorticotropic hormone (ACTH) that comes from the anterior pituitary. ACTH stimulates the adrenal gland and releases stress hormones such as corticosterone (Lucy et al., 2016). Behaviorally, ELS has been associated with less emotional and cognitive development (Baker et al., 2013).

THE IMPACT OF ELS ON BRAIN AND BEHAVIOR

To study the effects of developmental stress, researchers use early life stress (ELS) to look at biological and physiological changes following chronic and acute stressors (Herzberg et al., 2020). This is important because ELS can influence the development of many different disorders, such as irritable bowel syndrome by disrupting the relationship between our gut microbes, immunity, and the brain (O'Mahony et al., 2009). These stressful early experiences can cause abnormalities in a developing brain which leads to morphological changes in adulthood (Spinelli et al., 2009). Moreover, brain structures post-ELS have reported smaller brain sizes (Herzberg et al., 2020). Indeed, ELS can damage the hippocampus, resulting in a bigger amygdala, as well as increased anxiety, and learning and memory deficits (Eachus et al., 2021; Herzberg et al., 2020). In addition to brain changes, early stressors have a substantial impact on behavioral and hormonal responses (O'Mahony et al., 2009). For example, ELS can lower exploration time in a novel environment (Roman et al., 2006). Evidence of ELS affecting the brain has been linked to the prefrontal hippocampal amygdala circuits which play an important role in the release of CRH, emotional processing, memory, and learning (Hedges and Woon, 2011). Learning and memory are primarily controlled by the hippocampus, whereas emotional and social information is processed in the amygdala (Hanson et al., 2015). In rodents, there is decreased dendritic arborization in the hippocampus with similar findings in the amygdala. These changes are associated with depression and increased anxiety (Smith and Pollak 2020). Abnormalities due to ELS have been associated with mental and physical health issues as well as alterations in the circuits in the prefrontal-striatal dopaminergic which compromised the motivation which is due to the effects of cell death and proliferation (Smith and Pollak 2020). While a lot of research has shown similar results, prolonged ELS such as maternal separation has shown conflicting results.

Methods that are used for ELS vary greatly. Somes single-stressor for limited duration, other times multiple stressors across many days. For example, one study used a mild stressor on PND 65-75 (Lippmann et al., 200), whereas another used acute stressors on PND 33-35 (Lundbery et al., 2017). Therefore, ELS does not have a distinct definition, it often looks at the means of the strength and degree of severity of the manipulation. These methodological differences have made it difficult to generalize results.

In the current investigation, there I will focus n ambiguity in sex differences and the potential benefits of CBD for rats exposed to ELS.

AMBIGUITY IN SEX DIFFERENCES

Several studies have implicated that the way males and females respond to stress is different (Zareian et al., 2009). One study looked at leptin and found that there were sex differences and opposite effects on corticosterone levels in unstressed animals (Ahima et al., 1998). However, a similar study found no statistically significant differences between the unstressed animals, and the concentration of corticosterone was significant between male and female rats (Zareian et al., 2009). Maternal separation is commonly used as an early life stressor to study development in animal models, more specifically in rats (Daniels et al., 2009). This method was used because it causes long-term alterations in behavior such as aggression, and increased attacks such as pulling and biting (O'Mahony et al., 2009 & Veenema and Neumann 2008). Several studies have looked at sex differences and compared corticosterone levels with maternal separation, while some studies found there were differences between male and female rats, while other studies found no significant differences between the male and female rats.

One study explored the short-term effects of maternal separation (MS) on corticosterone levels in Wistar rats. The pups were separated from postnatal days (PND) 1-21 for either 15 or 360 minutes (Lundberg et al., 2017). In their research, they found that there were differences between male and female rats during the tests that were performed. The MS15 group showed no difference between the corticosterone levels of males and females. On the contrary, the MS360 rats showed significant differences. The main difference that was seen was that the male rats had more significant differences between the groups, while there was no difference between

the female groups (Lundberg et al., 2017). Another study looked at the long-term effect of maternal separation on Long Evan rats. The rats were separated on PND 2-14 and were subjected to either MS 15 or MS 180. Post an acute stressor, the maternally separated rats for 180 minutes had higher ACTH and corticosterone concentrations (Lippman et al., 2007).

POTENTIAL BENEFITS OF CBD FOLLOWING ELS

Cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) are both isolated from the cannabis plant known as *Cannabis sativa* more commonly known as marijuana (Withey et al., 2020 and Zou & Kumar, 2018). THC can lead to psychosis and impaired cognitive and motor functions, whereas CBD is non-intoxicating and does not generally cause cognitive impairment (Withey et al., 2020; Davies & Bhattacharyya, 2019). The mechanism of action for CBD is different from that of THC because it is an antagonist to the dopamine receptors (Davies and Bhattacharyya, 2019). There is a distribution of cannabinoid receptors (CB1) in the brain that plays a crucial role in the release of neurotransmitters (Jacobus and Tapert, 2014)

With the open field test, rats that underwent stress and were treated with CBD had an increased grooming time, locomotor, and exploration time. Corticosterone analysis from hair samples, CBD increased (Gàll et al., 2020). Female mice exposed to ELS found that CBD interferes with emotional memory, but improves behavior and cognitive impairment (Martín-Sánchez et al., 2022). This thesis will explore how early life stress affects rodents' development and corticosterone levels as well as the use of CBD as a potential therapeutic.

II. CURRENT INVESTIGATION

I chose these current investigation articles because they are recent articles within the past two years. These articles look at the questions this thesis investigates. The first article investigates the ambiguity of sex differences and fond there not to be sex differences. The second article investigates the potential use of CBD as a therapeutic in behavior and CORT.

Sex differences seen in studies indicate that males have changes in CORT levels within condition groups, but females do not have these specific alterations in CORT levels (Lundberg et al., 2017). Due to behavior alterations and CORT levels (O'Mahony et al., 2009), CBD was used to improve behavior (Martín-Sánchez et al., 2022). Current studies exhibit no sex difference (*Ceschim et al., 2021)* and that CBD has contradicting effects on behavior and CORT levels (*Gáll et al., 2020).*

II.I Maternally deprived male and female rats; found no sex differences (*Ceschim et al., 2021)*

This study used Wistar rats with a 24-hour maternal separation procedure on either postnatal day (PND) 3 or 11. The day the pups were born was assigned as postnatal day 0. Each of the litters was randomly assigned to one of the three experimental groups: control, separation on PND3, or PND11. The control liters were not handled, whereas the pups in the experimental group were separated from the dam for 24 hours. They maintained a clean environment by adding clean bedding that was placed on top of the heating pad to maintain a consistent temperature of 30-33°C. After the separation period ended, they were not disturbed. Post-weaning, the litters were divided by sex and the siblings were placed in the same cage. The rats had free access to chow and water and controlled the light-dark cycle and temperature.

To get basal conditions, four of the non-tested rats were used and the remaining two males and two females per litter were used for testing between PND 40 and 45. There were a total of 16 males and females per condition and group. Of the 16 rats per condition and group, six were decapitated to obtain plasma levels of corticosterone. Euthanization took place without anesthesia since it would alter the HPA axis activity. Post the social investigation test, the tested rats were euthanized, and non-tested rats were euthanized before their tested siblings. The other ten rats that were not subjected to testing were used for oxytocin immunoreactivity.

The rats were weighed before the open field test, and the average weight of the control rats was used as a reference. Habituation to the open field took place on PND 44 in a circular apparatus (figure 1) and the rats were placed at the center of the apparatus and were allowed to freely explore. Using Ethovision, they recorded the animals to measure anxiety-like behaviors, analyzing the time they spent in the center of the apparatus in

relation to the distance and time they spent moving around the arena. Increased exploration indicated less anxiety behaviors.

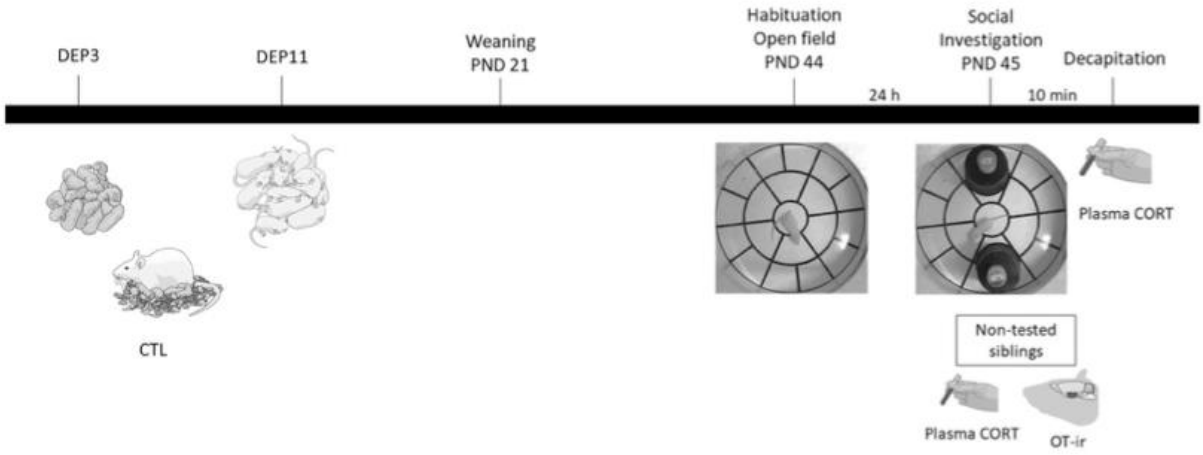

Figure 1: Summary of the experimental protocol. Rats were either separated on PND 3 or PND 11. Weaning took place on PND 21. On PND 44, the rats were habituated to the open field and social investigation was on PND45. Followed by decapitation and CORT plasma level assessment (Figure reproduced from *Ceschim et al., 2021).*

To increase their motivation for social interaction, the rats were isolated and individually housed post the open field test. They were then tested in a 10 minutes session where they used the same apparatus as the open field test with the addition of metal receptacles to avoid positional bias (Figure 1). For social stimulus, one of the receptacles contained the same sex and age as an unfamiliar rat, and the other receptacles were empty labeled as a non-social stimulus. Rats in this experimental group were placed in the center of the open field apparatus facing the wall and were recorded for behavioral analysis using XPlo-Rat software by an experimenter unaware of which groups the rats were in. Under the same conditions of habituation, to prevent olfactory clues, the apparatus was cleaned with 40% alcohol. Exploration time was calculated by the rats approaching the cage, climbing the cage, or reaching the top of the cage. To calculate the percentage of social preference they look at both the exploration of the social cage and the sum of time they explored both cages.

Corticosterone (CORT) plasma levels were determined using blood samples collected from the trunk. Samples were collected after the social interaction test. The tubes were chilled with EDTA and centrifuged for

15 minutes, and plasma was then extracted and stored. Using mass spectroscopy, CORT levels were determined. The remaining ten rats that were not subjected to testing were euthanized using sodium thiopental and using immunohistochemistry to test for oxytocin.

Analysis of the data was done separately based on the sex of the rat. CORT reactivity was obtained by the calculation of the individual values from the mean values of the non-tested rat group. CORT baseline percentage was analyzed using a one-way ANOVA.

Results of the body weights indicated that males and females on DEP3 were lighter than in control. Whereas the ones separated on DEP11 did not show any difference with the control or DEP3. Consequently, five males were not included in the behavioral analysis of the open field due to technical issues. There was no statistical difference between the male and female percentage of distance traveled. Findings of the social investigation test indicated that in both males and females there was no difference among the groups. However, DEP3 males were exploring the empty cylinder longer than the control and DEP11.

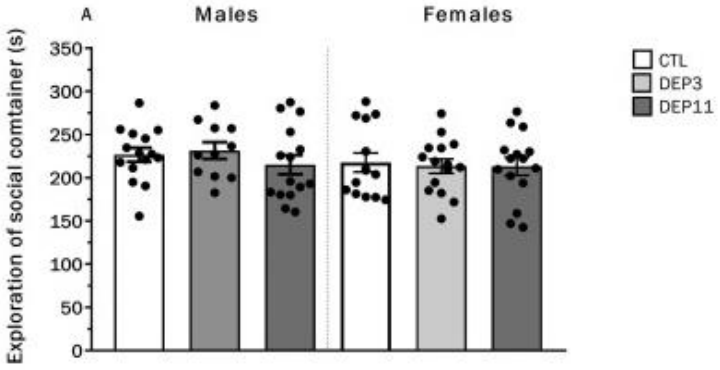

Figure 2: Time males and females spent investigating the social container, no difference was found in distance traveled between the male and female rats (Figure reproduced from *Ceschim et al., 2021).*

Social investigation in an open field was indicated by distance traveled. Results indicate that there was no difference found between the percent distance travel for males and females (figure 2).

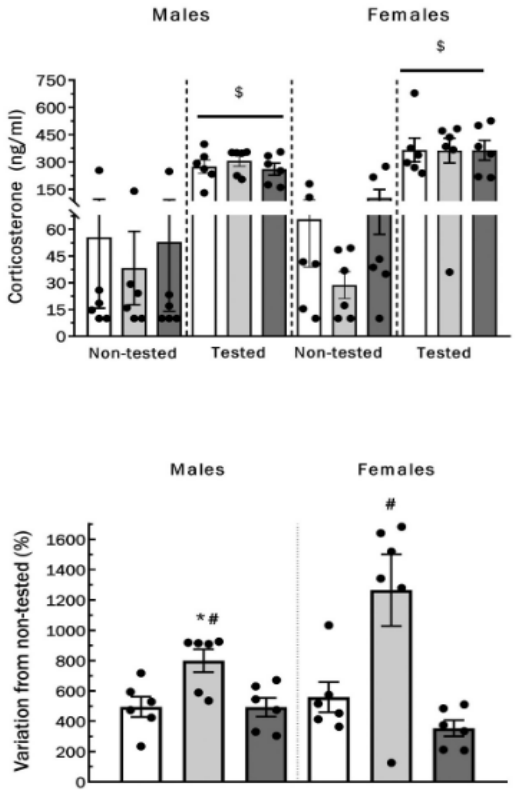

Figure 3: CORT levels post-social investigation. Showed an increase in baseline in both males and females (Figure reproduced from *Ceschim et al., 2021).*

CORT plasma levels showed there was no effect in the males, however, post the behavioral test, there were increased CORT levels. Percent analysis from the baseline showed that the reactivity of DEP3 was greater than the control or DEP11 (figure 3). Whereas, in females, there was an effect. Like the males, the females showed increased CORT post behavioral tests. Similar to the males, DEP3 females also showed high reactivity in the DEP3 group than those in the control and DEP 11 (figure 3).

The implication of these results is discussed in further detail in section III.II: ambiguity in sex differences.

II.II Chronic stress and how CBD affects behavior and CORT (*Gáll et al., 2020)*

This study looked at the effects of long-term use of cannabidiol (CBD) treatment with mild stress models of depression. Twenty-four male adult Wistar rats were exposed to a variety of daily stressors to induce anxiety-like behaviors. CBD was administered for 28 days by intraperitoneal injections. Multiple tests were

used to assess the effects of the CBD treatment. Hair samples were used for corticosterone assay by liquid chromatography-mass spectroscopy.

Wistar rats were habituated to handling and single housing for 21 days before the experiment started. The weekly weighing was recorded once or twice depending on whether stress factors included food deprivation. The rats were randomly divided into two groups, they were either placed in the vehicle control or CBD treated groups. Both groups received the treatment via the same injection protocol. A third group not subjected to stress was used to test CORT levels influence while given daily injections of vehicle.

Crystalline CBD was dissolved in saline that contained 4% dimethyl sulfoxide (DSMO) and 1% polysorbate 80 to be administered to the rats. In the acute experiment, the rats have injected a single dose with either vehicle or CBD 1 hour before the behavioral tests. Whereas, in the chronic experiment, the rat's CBD was administered daily based on body weight where they were given 10 mg/kg based on the body weight. Controls were injected with a mixture of saline, DMSO, and polysorbate 80.

Over four weeks, rats were exposed to chronic mild stress with various stressors (Table 1). Of the eleven stressors, two were applied each day at random. Behavioral assays were divided into three subgroups: sucrose preference test (SPT), open field test (OF), and elevated plus maze test (EPM). Chronic mild stress has been shown to decrease sucrose consumption. OF and EPM were performed on days 1 and 3 during the acute experiment, but only on days 29 and 31 in the chronic experiment to measure anxiety-like behaviors and locomotor activity.

Table 1: Chronic mild stressors used in the experiment (Table reproduced from *Gáll et al., 2020)*.

Stressors	Days of Experiments
Cage tilting	1, 6, 10, 17, 23, 27, 28
Damp bedding	2, 8, 12, 19, 26
Empty cage	5, 13, 20, 27
Cage swap	18
Foreign object	2, 9, 18
Water jet	12, 16, 22, 25
Paired housing	5, 23
Strobe flashing	3, 6, 11, 17, 20, 22
Hot air steam	4, 11, 16, 24
Food deprivation	6, 13, 20, 27
Water deprivation	4, 9, 15, 24

The SPT measured an anhedonia-like state only in the chronic experiment. The rats were given two bottles, containing 1% sucrose and the other bottle containing just water. 24 hours post-testing, the bottles were weighed, and data was collected. Compared to the total consumption, the ratio of the consumed sucrose was evaluated. The OF test was used to analyze anxiety-like behaviors. For five minutes, the animals were placed in the center of the testing arena and were recorded for analysis of their behavior. It was analyzed using EthoVision XT. They looked at the distance the rats moved, time spent in the center, and grooming activity. Following this, they ran an EPM test was run and recorded for five minutes. The plus-maze consisted of two open arms on opposite ends and two enclosed arms. To clean the maze in-between tests, they used 70% ethanol. Behaviors such as distance moved, time spent at each of the open and closed arms, rearing, head dipping, and the open arm preference were recorded.

Hair samples were used for the CORT assay before and after the chronic mild procedure. They shaved the rat's interscapular area to obtain samples without hair follicles. The hair samples were rinsed in methanol, dried for 24 hours, and crushed. The crushed samples were incubated in methanol. Using liquid chromatography-mass spectrometry, they were able to quantify CORT. To analyze all the data collected from the body weight, sucrose preference, and CORT, an ANOVA with one between subject and one within-subject factor were performed.

Results indicated the chronic use of CBD increased the body weight and the rats that underwent chronic stress showed a mean weight gain of 0.67%, whereas those exposed to chronic stress and CBD exhibited 5.94%. The SPT test was considered the baseline level at day 7 because at day 0 there was a strong neophobia response to the novel sucrose solution. There was no statistically significant preference in the chronic mild stress group. On the contrary, the rats with chronic mild stress and CBD showed an increase in sucrose preference, this showed that CBD affected the sucrose preference.

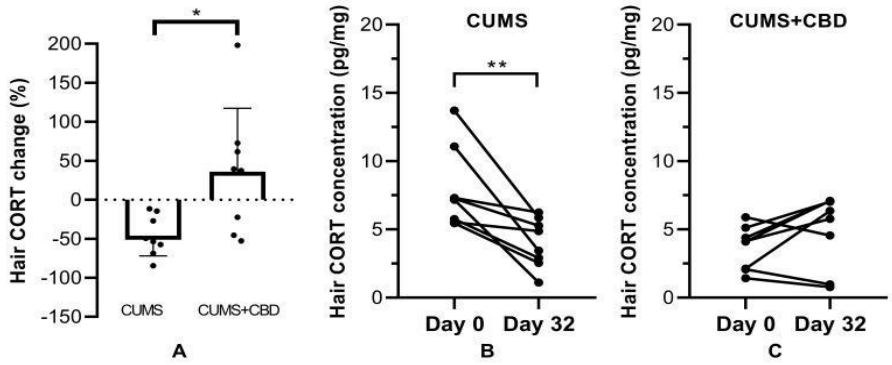

Figure 4: Hair CORT samples collected from day 0 and day 32, samples indicated that CBD increased CORT levels (Figure reproduced from *Gáll et al., 2020).*

Hair CORT showed no difference between the rats before and after isolation. Hair samples collected from the chronic mild stress group showed decreased levels compared to the baseline stressed groups. However, the CBD-treated groups showed an increase in hair CORT levels (figure 4).

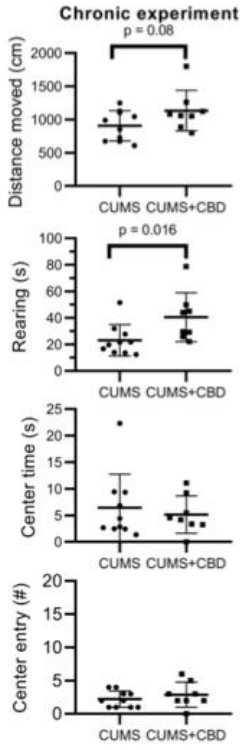

Figure 5: Chronic experiment results of the open field with vehicle treats in the first column and CBD treated rats in the second column (Figure reproduced from *Gáll et al., 2020).*

In the open field test, the chronically CBD treated group showed significance in anxiety-like behaviors from chronic stress. They showed increased exploration, distance moved, and rearing. The CBD treated group also had increased grooming time, locomotor, and exploration (figure 5).

The implication of these results is discussed in further detail in section III.II potential therapeutic benefits of CBD.

III. Discussion

Early life stress (ELS) is often used to study the effects of developmental stress (Herzberg et al., 2020). Evidentiary ELS has been found to impact the development of the brain, and immunity and has impacting effects on behavior (O'Mahony et al., 2009). Behavioral deficits can include lowered exploration time and cautiousness in a novel environment (Roman et al., 2006). The way males and females respond to stress is different (Zareian et al., 2009). While the current investigation found no sex differences in behavior and CORT assessment (Ceschim et al., 2021), whereas a separate study found sex differences (Lundberg et al., 2017). With the effects of ELS, researchers investigated the use of CBD as a potential therapeutic. The current investigation study looked at chronic unpredictable mild stress and the use of CBD as a way to cope with the effects of the stressors. Interestingly they found that CBD improved the behavioral effects of chronic stress, whereas it increased CORT levels (Gáll et al., 2020).

III.I Ambiguity in Sex Differences

Exposure to early life stress such as maternal separation suggests that there's an impact on brain development (Eachus et al., 2021 & Herzberg et al., 2020) as well as altering behavioral tendencies (Roman et al., 2006). Studies that found sex differences have implicated that females have lower CORT levels compared to the males post a stressor (Table 2) Moreover, studies using maternal separation differ in both the duration of separating pups from their mothers, as well as how many days the daily procedure is continued. Therefore, it is hard to generalize results due to many methodological differences.

Maternal separation as an early life stressor in Wistar rats found the males had significant CORT level differences between their male group conditions, whereas there were no significant differences between the

female groups (Lundberg et al., 2017). Both sexes were randomly assigned to a maternal separation condition either MS18 or MS360 on PND 1-21 as single isolation. Behavior and CORT levels were assessed, behaviorally, during a social play behavioral test, males in MS360 had higher pouncing and pinning tendencies than MS15 males and MS360 females. CORT levels had implicating results, male rats had clear differences between the condition groups. MS360 rats had higher baseline and CORT level recovery than those in MS15, whereas the female rats had no difference in the conditional groups (Lundberg et al., 2017).

On the other hand, a similar study using male and female Wistar rats found contradicting results. Prolonged maternal deprivation was used for a 24 hours period on either PND 3 or PND 11 (Ceschim et al., 2021). They also looked at behavior and CORT levels in both male and female rats. Using the open field test and investigation of both social and nonsocial containers to assess their behaviors, there were no differences indicated between the distance traveled in both sexes. Both sexes had increased exploration in the DEP3 compared to DEP11 and control. When assessing CORT levels, there were no differences between the sexes, DEP3 had increased levels than control and DEP11 (Ceschim et al., 2021). This is different from the previous study because no sex difference was found in behavior and CORT levels.

With differences in separation time and postnatal days, it is difficult to determine whether there are significant differences between male and female rats. As seen in Table 2, there are an insufficient amount of studies that look at both sexes: rather, the studies look at a variety of only male rat species. With not a lot of studies done on female rats, it is also harder to generalize the effects maternal separation has on them.

***Table 2*:** Summary table; highlighted references are used for general comparison

Reference	**Rat Species**	**MS Condition / Procedure**	**Sex Effects**	**CBD**
Aisa et al., 2007	Male Wistar Rats	Pups were separated from PND 2-14 for 180 minutes	X	X

Ceschim et al., 2021	Wistar Rats	Pups were separated on either PND 3 or PND 11 for 24 hours	X	X
Daniels et al., 2009	Male Sprague Dawley	Pups were separated from PND 2-14 for 180 minutes	X	X
Huot et al., 2002	Male Long Evans	Pups were separated from PND 2-14 for 15 and 180 minutes	X	X
Lehman, 2002	Male Wistar Rats	Pups were separated from PND 2-14 for 15 and 180 minutes	X	X
Lippman et al., 2007	Male Long Evans	Pups were separated from PND 2-14 for 180 minutes	X	X
Lundberg et al., 2017	Wistar Rats	Pups were separated from PND 1-21 for 18 and 360 minutes	M > F	X
Reese et al., 2006	Sprague Dawley	Pups were separated from PND 2-14 for 300 minutes	M > F	X
Roman et al., 2006	Male Wistar Rats	Pups were separated from PND 2-14 for 15 and 360 minutes	X	X
Roque et al., 2014	Male Wistar Rats	Pups were separated from PND 12-18 for 360 minutes	X	X
Veenema and Neumann, 2009	Male Wistar Rats	Pups were separated from PND 2-14 for 180 minutes	X	X

III.II Potential therapeutic benefits of CBD

Evidence suggests that cannabinoids (CBD) can be used to counteract the effects of early life stress. While a lot of research looked at how CBD affects behavior, the relationship to corticosterone is unclear. One study looked at how CBD affects maternally separated female mice. The mothers in this experiment were separated from their litters on PND 2-4 for 4 hours and on PND 6-16 for 8 hours. From PND 50-60 the female mice were given CBD intraperitoneally (Martín-Sánchez et al., 2022). The mice were then tested using three separate experiments. The first experiment examined whether CBD can be an antidepressant for maternally separated with early weaning (MSEW) mice using an elevated plus maze and the tail suspension test. The second experiment looked at how MSEW affects emotional memory with a passive avoidance test. Lastly, experiment 3 looked at the signaling pathways and mitochondrial damage using a western blot. Results showed that CBD had an impact on immobility time with a reduction compared to control mice. No alterations in emotional memory were observed through these experiments (Martín-Sánchez et al., 2022).

As previously discussed in the current investigation section, the study looked at the effects of CBD in chronic stress models of depression in rats, specifically observing hair corticosterone levels and observed behavior (Gáll et al., 2020). Results from this study indicated that CBD improved the behavioral aspect of chronic stress with increased distance traveled, weight gain, and sucrose preference. On the contrary, when analyzing CORT, there was an increase in levels (Gáll et al., 2020).

III.III Future directions

Moving forward there should be more consistency with the procedure to investigate the behavioral and CORT levels. There should be minimal methodological differences to evaluate whether or not there are sex differences. More studies should be done to evaluate how ELS affects female rodents. Most studies are done on male rats and therefore, it is harder to generalize the results obtained from the male rats with the female rats. There should also be research done to evaluate the therapeutic use of CBD following maternal separation. Studies have investigated the use of CBD with acute and chronic ELS, but not with maternally separated rodents.

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