**Effects of Cannabidiol on Social Cognition and Oxidative Stress Indices in a Social Isolation Animal Model of Schizophrenia**

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Conflict of Interest

I certify that there is no actual or potential conflict of interest or competing financial interest in relation to this article.

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Abstract

Objectives: Impairments in cognition and disruption of social behaviour in schizophrenia are associated with functional alterations in the prefrontal cortex and the striatum and oxidative stress induced by environmental and genetic factors. This study aimed to assess the effects of CBD on two main aspects of social cognition, namely, sociability and social novelty preference, social anxiety and oxidative stress parameters in the brain induced by post-weaning isolation.

Methods: A total of 40 male Wistar rats were randomly allocated to social isolation with or without pharmacological intervention or social rearing with or without pharmacological intervention. Rats received either CBD or distilled water for 16 days from Day 61 to Day 77. At the end of the isolation period, a 3-chambered social test was used to assess sociability or social affiliation, locomotion, and social novelty preference. The animals were sacrificed, and the homogenates from the harvested brain were used for assays of oxidative stress parameters.

Results: Social isolation affected locomotor activity and increased social anxiety; however social isolation did not influence social novelty preference in this study. Pharmacological intervention with CBD did not relieve social isolation-induced anxiety. Social isolation also increased nitric oxide (NO) activity, reduced glutathione (GSH) levels and reduced catalase (CAT) activity in the brain, CBD has effects on the oxidative stress enzymes induced by social isolation.

Conclusions: There was no evidence that CBD improved social isolation-induced anxiety. CBD did, however, ameliorate social isolation-induced oxidative stress.

Key Words: Cannabidiol; Social Isolation; Oxidative Stress; Antioxidants; Prooxidants.

**Introduction**

Schizophrenia is a neurodevelopmental disorder that can be associated with chronic and severe cognitive impairment (Bora 2015; Barron et al., 2017, Rees et al.,2021; Reckziegel et al.,2022). The onset of schizophrenia is usually insidious, preceded in most cases by cognitive symptoms, with positive, negative, and mood symptoms often later manifestations (Maas et al.,2017; Kaneko 2018; Kruk-Slomka et al.,2021; Mosolov et al., 2022 ). Social withdrawal, classified as a negative symptom, also frequently appears in the prodromal stage (Matheson et al.,2013; Stepnicki 2018 et al; Mouri et al.,2020; De Donates et al.,2022). While positive symptoms respond to medication, the relatively poorer response of negative and cognitive symptoms to medications contributes to the disability observed in patients with schizophrenia (Parnas, 2011; Foussias et al., 2014; Harvey et al.,2019; Rheenen et al.,2019). Significant progress has been made in the aetiology and pathophysiology of schizophrenia in general, though the pathophysiology of cognitive deficits is still poorly understood.

Cognitive symptoms of schizophrenia include impairments in attention and concentration, processing speed, verbal working memory, and reasoning (Keffe et al.,2012; Koola 2018). Investigators have mainly focused on the prefrontal cortex because of its importance in executive function and working memory; the striatum also has a major role to play in cognition (Balleine et al.,2007; Simpson et al.,2010; Báez-mendoza & Schultz 2013; Avram et al.,2019). The striatum is composed of three nuclei, caudate, putamen, and ventral striatum, which contain the nucleus accumbens (NAcc) (Hikosaka et al.,2000; Báez-Mendoza.,2013). The striatum and prefrontal cortex are tightly connected through GABAergic and cholinergic interneurons (Haber 2003; Calzavara et al.,2007; Chuhma et al.,2011; Báez-Mendoza.,2013 ).

Alterations in the integrity and function of the prefrontal cortex and the striatum can lead to cognitive deficits and disruption in social behaviour. As an example, hypofunction of the N-methyl-D Aspartate (NMDA) receptors reduced the activity of GABA interneurons in the prefrontal cortex but at a delayed rate, it also increases the firing rates of most of the pyramidal cells leading to decrease cortical inhibition of subcortical dopaminergic neurons and a decrease in dopamine release in the prefrontal cortex. Diminished prefrontal cortex dopamine is associated with cognitive and negative symptoms (Lewis & Lieberman, 2000; Lewis & Gonzalez-Burgos, 2006; Corlet et al., 2016; Tong et al., 2019). Working memory impairment, a key cognitive symptom, has been linked to reduced prefrontal dopaminergic transmission (Brozoski et al.,1979). Dysregulation of serotonergic and cholinergic neurotransmission within the prefrontal cortex is also associated with cognitive dysfunction (Hasselmo and Sarter 2011; Leiser et al., 2015; Koola 2016; Kim 2021).

Environmental stressors are directly associated with free radical production and oxidative stress linked to the neurotoxicity of the cellular membrane and mitochondrial damage (Yao et al., 2001; Prabakaran et al., 2004; Mhillaj et al., 2015). Oligodendrocyte precursor cells (OPC) are known to be susceptible to oxidative stress and OPC dysfunction has been linked to prefrontal cortex abnormalities in schizophrenia (Maas et al.,2017; Kolomeets et al., 2017). Environmental stressors can influence genes that control neurotransmitters involved in behavioural adaptation (Collier et al.,2003; Peñagarikano, 2007; Van Os et al., 2008; Howes et al., 2017; Guloksuz et al.,2019).

One of the aetiological hypotheses of schizophrenia is that multiple common genetic variations of small effect interact with environmental stressors to produce immunoinflammatory abnormalities and NMDA receptor hypofunction in the developing brain that in turn alters the function of the prefrontal cortex with consequent cognitive impairment and negative symptoms (Lewis & Lieberman, 2000; Redon et al., 2008; Van Os et al., 2008; Fatemi & Folsom, 2009; Laruelle, 2014; Guloksuz et al.,2019). As a result, individuals with schizophrenia may also have impaired social cognition which can be described as the inability to identify emotions, problems with sociability or social affiliation, difficulty in inferring people's thoughts,

and inappropriate emotional responses to others (Green et al., 2015; Kimoto et al., 2019). These social cognitive impairments interfere with social relationships and impair daily functioning in individuals with schizophrenia.

Current pharmacological treatment for schizophrenia, however, ameliorates mostly positive symptoms (Green et al.,2008; Green et al.,2015). The ideal drug for negative and cognitive symptoms should have antioxidant, anti-inflammatory, and immunomodulatory properties and be active at the dopaminergic, serotonergic, and glutaminergic receptors. Cannabidiol (CBD) is a phytocannabinoid from the plant *Cannabis sativa* (Booz, 2012; Oliere et al., 2013) and modulates the activity of the endocannabinoid system (ECS) (Badala et al., 2010). The ECS is a broad neuromodulatory system that is involved in synaptic plasticity and the modulation of endogenous cannabinoids. The ECS is made up of cannabinoid receptors (CB1, CB2), peroxisome proliferator-activated receptors (PPARs), transient receptor potential channels (TRP), endocannabinoids (e.g. 2-arachidonoyl glycerol (2-AG) and anandamide) (Lu & MacKie, 2016; Zou & Kumar 2018).

The ECS is important in the body’s response to oxidative stress, inflammation, and modulation of the immune system (Booz et al., 2012; Gallegos, 2015). CBD exerts its antioxidant, anti-inflammatory, immunomodulatory, and therefore neuroprotective effects through its interaction with the ECS. It is a partial agonist at the CB2 receptor, and it also inhibits the degradation of anandamide by fatty acid amide hydrolase (FAAH) (Rohleder et al., 2016; Karhson et al., 2016; Leweke et al., 2018). CBD also modulates the mesolimbic dopaminergic system by partially antagonising D2 receptors. High D2 receptor affinity contributes to the functional effects of dopamine (Sonego et al., 2018). CBD also binds to the 5HT1 receptor system to produce its effects on the positive symptoms of schizophrenia (Seeman, 2016; Norris et al 2016; Renard et al., 2017) and modulates the brain’s excitatory glutamatergic system to ameliorate negative symptoms (Mandolini et al., 2018; Pretzsch et al., 2019; Szkudlarek et al., 2019).

Preclinical and clinical studies have reported on the antipsychotic effects of CBD, though the mechanism of action by which it produces its actions is still poorly understood (Deina, 2013; Devinsky et al., 2014; Steeds et al., 2015; Cripa et al., 2015; Peres et al., 2017; Batala et al., 2019). Since cognitive and negative symptoms remain the major challenge in the management of schizophrenia, it is important to focus more on these symptoms and determine cannabidiol’s effectiveness in the treatment of these symptoms in animal models. There are however few studies in this area (Peres et al., 2016a & 2016b; Osborne et al., 2017). A spontaneously hypertensive rat (SHR) model of schizophrenia was used to study the effects of CBD on locomotor activity, social interaction, pre-pulse inhibition (PPI) of startle, and contextual fear conditioning, CBD halted the emergence of hyperlocomotion and PPI deficits in the SHR strain but did not attenuate impaired social interaction (Peres, 2018). Others have used the Poly I-C prenatal infection model to study the effects of CBD on working memory, social interaction, and recognition and have found in this model that CBD attenuates social interaction and cognitive deficits (Osborne et al., 2017).

It is necessary to extend the investigation of the effects of CBD on negative and cognitive symptoms using other models, to improve our knowledge of negative and cognitive deficits in schizophrenia. In this study, we assessed three parameters: social affiliation or interaction, social novelty preference, and locomotor activity (an indicator of social anxiety and social withdrawal), using a stress protocol that translates to early life adversity in humans (Fones & Porkess, 2008; Li et al., 2017). This study aimed to measure the effects of CBD on social cognition, locomotor activity, and oxidative stress parameters in the brain induced by post-weaning isolation.

**Methods**

Experimental procedures were undertaken in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (National Research Council, 2011). The study is a component of a larger study approved by the Institutional Review Board (IRB) of NIMR, Yaba, Lagos, Nigeria (IRB/16/329) and Stellenbosch University’s Health Research Ethics Committee: Animal Care and Use (SU-ACUD16-00137).

A total of 40 male Wistar rats bred and housed at the Nigerian Institute of Medical Research, Yaba, Lagos animal house was used. The social isolation-rearing protocol was described by Regenass et al. (2018). The rats were weaned (separated from their mothers) at Postnatal Day (PND) 21 which still falls within the range of normal standard weaning between PND 20 and PND 25 (Curley et al., 2009). The weaned animals were then removed from their home cages and randomized to either socially reared (3 rats/cage) or isolation-reared rats (1 rat/cage). Ten animals were assigned to each group. The animals were in their new homes for 8 weeks till PND 77 and received either CBD or distilled water for 16 days from PND 61 till PND 77 (Möller et al., 2012, 2013a). Early antipsychotic response in schizophrenia, at 14 days, is reported to be an accurate predictor of symptom response (Kapur et al., 2005; Ascher-Svanum et al., 2008). Other researchers have used between 14 to 16 days of medication administration in social isolation studies (Möller et al.,2013). Handling of the animals was avoided as much as possible to reduce the confounding effects on social isolation in the study. Male adult Wistar rats used in this study were obtained from the Nigerian Institute of Medical Research (NIMR) colony, Yaba, Lagos, Nigeria. The animals were kept in clean polypropylene cages in well-ventilated and hygienic compartments, maintained under standard environmental conditions, and fed with standard rodent pellets (Ladokun Feed Plc., Ibadan, Nigeria) and water *ad libitum.*

The first group of socially isolated rats received only 5 mg/kg/oral CBD from PND 61 for 16 days.

The second set was socially reared rats and received 5mg/kg/oral of CBD from PND 61 for 16 days.

The third set of socially isolated animals did not receive any medication but distilled water (2 ml) for the 8 weeks (PND 77) of social isolation and at the end of isolation.

The fourth set was socially reared rats and did not receive any medication except distilled water (2 ml), for the duration of the experiment.

The sociability apparatus (3-chambered social test) was used to assess the frequency and duration of contact with stranger 1 which measures social affiliation or social interaction, frequency of line crossings which measures locomotion (Blanchard et al., 2001), and frequency and duration of contact with stranger 2 which measures social novelty preference in rodents.

*Equipment*

The sociability apparatus consists of a 3-chambered cage, with grey opaque walls, a special non-reflective, grey-coloured floor, and 2 grid enclosures. This three-chamber paradigm test known as Crawley's sociability and preference for social novelty protocol has been successfully employed to study social affiliation and social memory/social novelty preference in several inbred and mutant mouse/rat lines (Clapcote et al., 2007; Labrie et al., 2008; Kaidanovich-Beilin et al., 2009).

The main principle of this test is based on free choice by a subject rat (i.e., the experimental rat whose behaviour is being studied) of spending time in any of three boxes compartments during two experimental sessions, including indirect contact with either the first control (Stranger 1) and the second control (Stranger 2). The subject rat can only contact either of the control rats through the metal wire of the container that houses the control rat. To quantify the social tendencies of the experimental rat, the main tasks are to measure a) the time spent with a novel conspecific and b) preference for a novel vs. a familiar conspecific. (Familiar conspecific is stranger 1 and novel conspecific is stranger 2). Thus, the experimental design of this test allows the evaluation of two critical but distinguishable aspects of social behaviour, namely social affiliation/motivation and social memory and novelty. "Sociability" or social affiliation in this case is defined as the propensity to spend time with another rat, as compared to time spent alone in an identical but empty chamber (Moy et al.,1992).

*Room Set-Up*

1. The apparatus for Crawley's sociability and preference for social novelty test comprises a rectangular, three-chamber box. Each chamber is 19 × 45 cm, and the dividing walls are made from clear Plexiglas, with an open middle section, which allows free access to each chamber. The floor is made of white painted wood and divided by permanent read markings into 16 equal squares at the bottom. The number of line crossings was used to assess locomotor activity.

2. Two identical, wire cylinder-like containers with removable lids that are large enough to hold a single rat were used. These were placed vertically inside the apparatus, one in each side chamber, to contain the control rats (either stranger 1 or 2). These rats were randomly picked from another group, although age, weight, and sex were similar to the subject rats. Each container is comprised of metal wires to allow for air exchange between the interior and exterior of the cylinder but is small enough to prevent direct physical interactions between an animal on the inside with one on the outside.

3. Behavioural testing was performed between 9:00 am and 6:00 pm.

4. General room lighting was about 650 lux.

5. Two independent observers monitored and recorded all the behavioural parameters assessed. After each trial, all chambers were cleaned with 70% ethanol (between rats) and then with Clidox to prevent olfactory cue bias and to ensure proper disinfection, respectively (Kaidanovich-Beilin et al., 2011).

*Procedures*

Two sets of rats were used for these experiments, a control, naïve, or "unfamiliarised" animal and the test subject (socially isolated rats/socially reared rats). For the control, we used a rat of the same background age (usually 8-12 weeks old), gender, and weight, without any prior contact (not littermates) with the subject rats. Two control rats were used per experiment, one for the session I and another for session II. The same control rats could be used between trials.

At the start of each experiment, the subject rat was placed at the centre of the middle chamber/compartment to habituate for 5 minutes.

Social Affiliation Aspect of the Test (Session I):

1. One of the control rats ("Stranger 1") was placed inside a wire containment cup that was in one of the side chambers. The placement of Stranger 1 on the left or right side of the chamber was systematically altered between trials.

2. The walls were removed between the chambers, to allow free access for the subject rat to explore each of the three chambers.

3. Immediately, the observers began to monitor and record the following parameters:

3.1 Duration and number of direct (active) contacts between the subject rat and the containment cup housing the Stranger 1 rat and the empty containment were scored, for each chamber individually. Direct contact between the subject rat and the containment cup or stretching of the body of the subject rat in an area 3-5 cm around the cup was counted as active contact.

3.2 Locomotor activity was assessed by the frequency of line crossings in each chamber. The duration of session 1 was 10 minutes.

Social Novelty/Preference Session of the Test (Session II):

1. A second control rat ("Stranger 2") was placed inside an identical wire containment cup in the opposite side chamber (that had been empty during Session I). The same parameters described above were monitored (duration of contact, number of contacts, and locomotor activity), differentiating the behaviours between the subject rat in the presence of Stranger 1 compared with Stranger 2. The duration of Session II was also 10 minutes**.**

*Antioxidant assays*

After the behavioural experiments were concluded, the animals were sacrificed by first anaesthetizing with 50mg/kg i.p. phenobarbitone before cervical dislocation and dissection by opening the abdomen. The brains of the rats were isolated and dissected on ice. 10% w/v of the brain sample (0.03 M sodium phosphate buffer, pH 7.4) was homogenized. The homogenates generated from processed tissues were then used for oxidative stress indices, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and nitric oxide (NO) assays.

The following antioxidant indices were determined spectrometrically:

Malondialdehyde (MDA) is an index of lipid peroxidation which was assayed using the method of Buege and Aust (1978)

1. 1 ml of tissue homogenate was combined with 2 ml of tri-carboxylic acid (TCA)- thiobarbituric acid (TBA)- hydrochloric acid (HCl) reagent and mixed thoroughly.

3. The solution was heated for 15min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 revolutions per minute for 10 minutes.

4. The absorbance of the supernatant was measured at 532 nm against a blank that contains all the reagents minus the homogenate. The malondialdehyde concentration of the sample was calculated using an extinction coefficient of 1.56 × 105 M-1CM-1Malondialdehyde concentration (M) = Absorbance at 532nm / 1.56 x 105.

The tissue's reduced glutathione (GSH) content as non-protein sulfhydryl was estimated according to the method described by Sedlak and Lindsay (1968). To the homogenate, 10% TCA was added and then centrifuged. 1.0 ml of supernatant was treated with 0.5ml of Ellman’s reagent (19.8 mg of 5, 5-dithiobisnitrobenzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nanometers (nm), Ʃ =1.34 × 104 M-1 centimetre(cm)-1

Catalase activity was determined according to the method of Sinha (1972). It was assayed colourimetrically at 620 nm and expressed as micromoles (µmol) of H2O2 consumed/min/mg protein at 25ºC. The reaction mixture (1.5 ml) contained 1.0 ml of 0.01Mole phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate, and 0.4 ml of 2 Mole (M), H2O2. The reaction was stopped by the addition of 2.0 ml of dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in a 1:3 ratio). Ʃ =40 M-1cm-1.

Superoxide dismutase activity was determined as described by Sun and Zigma (1978). The reaction mixture (3 ml) contained 2.95 ml 0.05M sodium carbonate buffer pH (10.2), 0.2 ml of tissue homogenate, and 0.03 ml of epinephrine in 0.005 normal(N) HCl was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of the substrate (epinephrine), and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 minutes Ʃ =4020 M-1cm-1.

*Nitric oxide (NO) scavenging activity*

A volume of 2 ml of sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of our tissue homogenate at various concentrations (0.2-1.0 mg/mL). The mixture was incubated at 25 °C for 180 minutes. An aliquot of 0.5 ml of the solution was added to 0.5 ml of Griess reagents [(1.0 ml of sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5 minutes with 1 ml of Naphthyethylenediamine chloride (0.1% weight/volume (w/v))]. The mixture was incubated at room temperature for 30 minutes. The absorbance (Abs) was then measured at 540 nm. The amount of nitric oxide radical was calculated using the equation:

NO radical scavenging activity = [(Abs control- Abs sample)/ (Abs control)] /100, where Abs control is the absorbance of NO radical + methanol; Abs sample is the absorbance of NO radical + sample tissue homogenate or standard.

*DPPH scavenging assay*

A portion of 0.135 mM DPPH was prepared in methanol containing 0.5 mg of the tissue homogenate and standard drug (Butylated hydroxytoluene (BHT) and Rutin). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the tissue homogenate on DPPH was calculated using the equation:

DPPH scavenging activity (%) = [(Abs control – Abs sample)]/ (Abs control)] /100, where Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + tissue homogenate or standard. IC50 = tissue homogenate concentration that yields half the maximum free radical scavenging activity.

**Statistical analysis**

Data were analysed using the IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp). Descriptive statistics of relevant study variables and parameters from behavioural and biochemical assays were determined using means and standard deviations (SD) as measures of central tendency and dispersion for individual groups. When data were not normally distributed results were presented as median (interquartile range). A comparison of the equality of means between groups was run using a one-way ANOVA test. Where the data were not normally distributed, a comparison of medians was done using the Kruskal-Wallis test. When the F statistic was significant (<0.05), depending on the violation of the homogeneity of variance, Tukey’s HSD test or Games Howell post hoc tests were run to determine the differences between the groups.

**Results**

*Number and duration of contacts with stranger 1 and empty cylinder*

In the first stage of the experiments (Session 1), there was a significant difference in the number of contacts with stranger 1 (p=0.000) when the means of the four groups were compared with each other. There was also a significant difference in the number of contacts with the empty cylinder (p=0.029) when the means of the four groups were compared with each other and a significant difference in duration of contact with stranger 1 when the means of the groups were compared with each other (p=0.000). We also observed a significant change in the duration of contact with the empty cylinder (p=0.003) when the means of the groups were compared with each other (Table 1).

**Table 1: Number and duration of contacts with stranger vs. empty cylinder**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **Mean ± SD** | **F**  **P-value** | **Mean ± SD** | **F**  **p-value** |
| **Number of contacts** | *Stranger1* |  | *Empty* |  |
| Group A (Isolated +CBD) | 15.7 ± 5.8 |  | 7.7 ± 4.1 |  |
| Group B (Reared +CBD) | 8.3 ± 5.7 | F=8.867 | 3.5 ± 3.2 | F=3.388 |
| Group C (Isolated+ Distil water) | 7.4 ± 2.0 | P < **0.001** | 4.8 ± 1.4 | P = **0.029** |
| Group D (Reared + Distil water) | 6.8 ± 2.0 |  | 4.8 ± 2.7 |  |
| **Duration of contacts** |  |  |  |  |
| Group A (Isolated +CBD) | 105.6 ± 46.4 |  | 61.0 ± 34.5 |  |
| Group B (Reared +CBD) | 49.8 ± 40.1 | F = 6.488 | 19.0 ± 17.1 | **F=5.610** |
| Group C (Isolated+ Distil water) | 127.8 ± 34.2 | P = **0.001** | 60.8 ± 24.5 | **P=0.003** |
| Group D (Reared + Distil water) | 111.3 ± 43.6 |  | 43.5 ± 22.8 |  |

A pairwise comparison was then done to localize the differences within the groups and observe if there were differences among the isolated and reared rats for each pharmacological intervention (i.e Groups A-B and Groups C-D). The results are presented below:

*Number of contacts with stranger1:*There were significant differences between Groups A and B (p=0.004), A and C (p=0.001), and A and D (p<0.001).

*Number of contacts with empty cylinder:* There were significant differences between Groups A and B (p=0.024).

*Duration of contact with stranger 1:*There were significant changes between groups A and B (p=0.006), B and C (p=0.001), and B and D (p=0.013).

*Duration of contact with empty cylinder:*There were significant differences betweenGroups A and B (p=0.006), B and C (p=0.008) (Table 1).

*Number and duration of contacts with stranger 1 vs. stranger 2*

There were no significant group differences in the number of contacts with stranger 1 (p=0.29) and 2 (p= 0.522). There was a significant difference in the duration of contact with stranger 1 (p=0.010) but not with stranger 2 (p=0.234) (Table 2). Pairwise comparison of the duration of contact with stranger 1 revealed the difference was between groups B and D.

**Table 2: Number and duration of contacts with stranger 1 vs. stranger 2**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **Mean ± SD** | **F**  **P-value** | **Mean ± SD** | **F**  **p-value** |
| **Number of contacts** | *Stranger1* |  | *Stranger 2* |  |
| Group A (Isolated +CBD) | 5.6 ± 5.5 | F=1.509 | 5.2 ± 7.3 | F=0.764 |
| Group B (Reared +CBD) | 2.5 ± 4.0 | P=0.29 | 3.0 ± 2.4 | P=0.522 |
| Group C (Isolated+ Distil water) | 2.3 ± 2.2 |  | 2.8 ± 2.2 |  |
| Group D (Reared + Distil water) | 3.6 ± 2.7 |  | 2.9 ± 2.1 |  |
| **Duration of contacts** |  |  |  |  |
| Group A (Isolated +CBD) | 27.3 ± 24.2 | F=4.408 | 25.1 ± 37.4 | F=1.492 |
| Group B (Reared +CBD) | 17.7 ± 24.8 | P=**0.010** | 24.9 ± 20.0 | P=0.234 |
| Group C (Isolated+ Distil water) | 34.3 ± 40.1 |  | 62.4 ± 64.0 |  |
| Group D (Reared + Distil water) | 73.2 ± 51.0 |  | 101.6 ± 171.4 |  |

**Locomotor activity**

*Frequency of line crossings with stranger 1 and empty cylinder*

There was a statistically significant difference in the frequency of line crossings between the groups with stranger 1 (p <0.001). There was also a statistically significant difference in the frequency of line crossings between the groups with the empty cylinder (p <0.001) (Table 3).

*Frequency of line crossing with stranger 1 and stranger 2*

There was a statistically significant difference in the frequency of line crossings between groups with stranger 1 (p=0.002), but no appreciable difference between the groups with stranger 2 (p= 0.050) (Table 3).

**Table 3: Frequency of line crossing (Locomotor activity)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **Mean ± SD** | **F**  **P-value** | **Mean ± SD** | **F**  **p-value** |
| **Frequency of Line crossings** | *Stranger 1* |  | *Empty* |  |
| Group A (Isolated +CBD) | 19.0 ± 4.8 |  | 51.6 ± 13.4 |  |
| Group B (Reared +CBD) | 12.3 ± 9.8 | F = 20.119 | 43.2 ± 36.1 | F = 12.145 |
| Group C (Isolated+ Distil water) | 13.8 ± 4.3 | P **< 0.001** | 40.9 ± 15.5 | P **< 0.001** |
| Group D (Reared + Distil water) | 51.0 ± 22.3 |  | 0.0 ± 0.0 |  |
| **Frequency of Line crossings** | Stranger 1 |  | Stranger 2 |  |
| Group A (Isolated +CBD) | 8.3 ± 9.5 |  | 28.7 ± 39.4 |  |
| Group B (Reared +CBD) | 7.1 ± 5.8 | F = 6.113 | 26.7 ± 24.2 | F = 2.878 |
| Group C (Isolated+ Distil water) | 7.2 ± 4.3 | P = 0.002 | 16.7 ± 14.5 | P = 0.050 |
| Group D (Reared + Distil water) | 29.6 ± 25.1 |  | 0.0 ± 0.0 |  |

*Frequency of line crossings with stranger 1 and empty cylinder (Pairwise comparisons)*

*Frequency of line crossings with stranger 1:*Pairwise comparison revealed differencesbetween groups A and D (p = 0.012), B and D (p=0.003) and C and D (p=0.004). There were no significant differences between A and B (p=0.424).).

*Frequency of line crossings with empty cylinder:*Pairwise comparison revealed differencesbetween groups A and D (p=0.000), B and D (p=0.036), and C and D (p=0.000). There were no significant differences between A and B (p=0.980).

*Frequency of line crossings with stranger 1 and stranger 2 ( Pairwise comparison)*

*Frequency of line crossings with stranger 1:*Pairwise comparison revealed differencesbetween groups A and D (p=0.007), B and D (p=0.003), and C and D (p=0.005).

*Frequency of line crossings with stranger 2:*No significant difference was observed.

*Brain oxidative stress indices after the last dose of pharmacological intervention*

We compared the results of the oxidative stress indices of the six groups in the brain and observed significant and non-significant differences. MDA (p=0.211), CAT (p=0.000), SOD (p=0.081), GSH (p= 0.000), DPPH scavenging activity (p=0.000), and NO (p=0.000) (Table 4).

**Table 4:** **Brain oxidative stress indices**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Brain antioxidant indices** | **Group A**  Mean ± SD | **Group B**  Mean ± SD | **Group C**  Mean ± SD | **Group D**  Mean ± SD | **Group E**  Mean ± SD | **Group F**  Mean ± SD | **F** | ***p-value*** |
| CAT (µmol/ml/min/mg pro) | 22.3 ± 15.7 | 44.8 ± 24.9 | 21.1 ± 14.2 | 38.9 ± 13.0 | 10.1 ± 2.8 | 40.8 ± 22.7 | 6.230 | **0.000** |
| GSH (µmol/ml) | 10.0 ± 12.1 | 10.6 ± 13.0 | 22.5 ± 16.0 | 10.7 ± 8.4 | 43.2 ± 21.2 | 10.1 ± 5.9 | 8.781 | **0.000** |
| NO (µmol/dl) | 16.1 ± 2.3 | 16.1 ± 4.0 | 18.3 ± 2.9 | 16.9 ± 4.5 | 23.6 ± 5.5 | 15.6 ± 4.6 | 4.987 | **0.001** |
| MDA (µmol/ml) | 3.9 ± 2.3 | 5.7 ± 1.8 | 3.8 ± 3.6 | 5.9 ± 2.0 | 4.4 ± 1.8 | 5.4 ± 2.0 | 1.482 | 0.211 |
| SOD (µmol/ml/min/mg pro) | 5.4 ± 5.3 | 4.6 ± 2.4 | 2.8 ± 1.9 | 4.1 ± 1.7 | 1.9 ± 0.7 | 4.9 ± 2.0 | 2.307 | 0.081 |
| % Scavenging activity in DPPH (µg/ml) | 75.3 ± 3.5 | 43.4 ± 14.2 | 72.7 ± 5.2 | 40.5 ± 15.9 | 63.9 ± 8.4 | 45.3 ± 16.1 | 17.736 | **0.000** |

*Brain GSH:* Pairwise comparisons revealed significant differencesbetween Groups A and E (p=0.013), B and E (p=0.015), C and F (p=0.013), and E and F (p=0.012). There were no differences between Groups A and B (p=1.00): C and D (p=0.356).

*Brain CAT:* Pairwise comparisons revealed significant differencesbetween Groups B and E (p=0.015), D and E (p=0.001), and E and F ( p=0.018). There was no difference between Groups A and B (p=0.212) and C and D (p=0.082).

*Brain DPPH:* Pairwise comparisons revealed significant differencesbetween Groups A and B (p=0.000), A and D (p=0.001), Group A and E (p=0.030), A and F (p=0.002), B and C (p= 0.001), B and E (p=0.016), C and D (p=0.001), C and F (p= 0.003), and D and E (p= 0.012)**.** There was no significant difference between E and F (p=0.057)

*Brain NO:* Pairwise comparisons revealed significant differencesbetween Groups A and E (p=0.030), B and E (p=0.043), and E and F (p=0.034). There were no differences between Groups A and B (p=1.000) and C and D (p=0.692).

**Discussion**

In this study, we examined the effects of CBD on social affiliation, locomotion, novelty preference, and oxidative stress parameters using a post-weaning social isolation model of schizophrenia. Our results indicate that social isolation alone did not affect social affiliation/interaction of the animals but reduced locomotion in the presence of stranger 1. This study also revealed that social isolation influenced some oxidative stress indices when the oxidative stress indices of socially isolated and socially reared rats administered the same pharmacological interventions were compared.

Our results indicate that isolation alone did not affect the social affiliation of the animals because Group C (Isolated+ Distilled water) and Group D (Reared + Distilled water) did not reveal any significant difference, both in the number and duration of contacts between them in respect of contact with empty cylinder and stranger 1. There was also no significant difference in the number and duration of contacts with strangers 1 and 2 on the social novelty or memory test. These findings together indicate that neither CBD nor social isolation influences social cognition. Our findings on the influence of social isolation on cognition concur with earlier investigators who reported similar behaviours between isolated and group-reared rats (Gardner et al., 1975; Gamallo et al.,1986; Fulford & Marsden, 1998a,b; Weiss et al., 2004) and a recent study who found no strong or consistent effects of single housing on measures of anxiety in the open field, elevated plus maze, and social behaviour (Bailoo et al.,2020), but contradicts other studies who found an association between social isolation and impaired social cognition (Lukkes et al., 2009; Brandão et al.,2015; Song et al., 2021).

Differences in the findings from social isolation studies may be attributed to methodological differences, such as social isolation ( SI) procedures, test environment and the strain of rats used (Einon and Morgan 1977; Einon and Morgan 1978; Weiss et al.,2000) and important methodological differences that contribute to varying results include differences in study design, such as weaning age, strain and sex of animals, methods of individual housing, cage sizes, flooring conditions contribute to varying results between studies (Krohn et al., 2006).

There are very few studies on the effects of CBD on behaviour in social isolation experiments in rodents. Our observation that CBD improved social affiliation is consistent with Hartmann et al. (2019) who described the calming effect of CBD on aggressive behaviours induced by social isolation in rats in their study.

We assessed locomotion using Crawley's sociability apparatus as a modified open-field apparatus. Group C (Isolated + Distilled water) had significantly less locomotor activity than Group D (Reared + Distilled water) in the compartment with Stranger 1 and more activity than Group D in the compartment with the empty container. These observations suggest that social isolation interfered with locomotion in the presence of a stranger. Our results also suggest that this finding was not ameliorated by CBD alone. We also assessed locomotion in compartments with stranger 1 and 2. There was a significant increase in locomotor activity of Group D (Reared + Distilled water) compared to other groups, indicating the normally reared rats without any intervention did better with Stranger 1, while the isolated rats performed relatively poorly. There was however no difference among the groups in the compartment with Stranger 2. Our experiments on locomotion indicated that social isolation increased anxiety and social withdrawal and CBD at 5 mg/kg alone or combined with haloperidol did not relieve anxiety and social withdrawal induced by social isolation. Our observations also suggest that CBD alone or combined with haloperidol did not improve locomotion in the presence of the second stranger.

Investigators have reported different effects of isolation rearing on locomotion. Most studies reported hyperactivity in socially isolated male rats (Heidbreder et al., 2000; Zhao et al., 2009; Skelly et al., 2015), while some did not find any change in locomotion (Weiss et al., 2004; Thorsell et al., 2006). Others showed that isolation led to hypoactivity (Holson et al., 1988; Holson et al., 1991). Our findings are consistent with those of the last-mentioned authors. Differences in study design, duration of isolation and environmental conditions may be contributing to differences in findings across studies (Walker et al., 2019).

Social isolation in rodents is associated with neurobiological and neurochemical abnormalities (e.g., a hyperactive mesolimbic dopaminergic system). Enhanced presynaptic dopamine (DA) and serotonergic (5-HT) function in the nucleus accumbens (NAcc), hypofunction of mesocortical DA, and attenuated 5-HT function in the prefrontal cortex and hippocampus are also linked to social isolation. All these abnormalities are also found in schizophrenia (Fones and Porkess, 2011; Winton-Brown et al., 2014; Li et al., 2017). These neurobiological and neurochemical abnormalities induced by social isolation may have been responsible for the phenotypic changes we observed in our study.

*Effects on brain antioxidant indices*

For GSH, there was a statistically significant difference in level between Group C (Isolated + Distilled water) and Group D (Reared + Distilled water), with Group C having a higher level. There was no significant difference between Groups A (Isolated + CBD) and B (Reared + CBD). CBD may have ameliorated the effects of a deranged redox system and this could explain why we did not observe a difference between Groups A (Isolated + CBD) and B (Reared + CBD).

Our study contradicts that of Zlatkovic et al. (2014) who observed depletion of GSH in isolated rats, though our experimental designs were different. However, the findings are in the same direction as that of Djordjevic et al. (2010) who reported that long-term isolation decreased the activity of GPx.

Even though the pair of Group C (Isolated + Distilled water) and Group D (Reared + Distilled water) reflected a decrease in SOD in the isolated groups, differences between the groups were not significant. Moller et al. (2011) and Pajovic et al. (2006) reported an increase in SOD activity in response to long-term isolation. Zlatkovic et al. (2014) described decreased total SOD activity in the prefrontal cortex of rats exposed to chronic stress. We are unable to find a study on the role of CBD in reversing oxidative stress induced by social isolation; more studies will be needed in this direction.

Social isolation appears to have reduced CAT activity in this study because the socially isolated groups had lower CAT activity than the socially reared groups with the same interventions, with Group C (Isolated + Distilled water) having the least activity though this was only significant between Groups C (Isolated plus distilled water) and D (Reared plus distilled water). The reduced CAT activity in the socially isolated groups suggested social isolation-induced oxidative stress with increased free radical production, in the isolated groups and CBD may have ameliorated the oxidative stress in Groups A (Isolated + CBD) and increased CAT activity, thereby reducing the differences in CAT activity between Groups A (Isolated + CBD) and B (Reared + CBD).

The low CAT activity in social isolation observed in our study was reported by other workers (Krolow et al., 2012; Pejic et al., 2016; Famitafreshi and Karimian, 2019). Djordjevic et al. (2010) did not observe any change in CAT activity after chronic social isolation. CBD was found to augment the effect of CAT in the presence of oxidative stress by several investigators (Sajjadian et al., 2017; Briggs 2018; Sonego 2018), but we are not aware of any study on the effect of CBD on CAT in a social isolation model.

There was no evidence that social isolation induced lipid peroxidation in the brain in this study because we did not observe any difference in MDA levels. D'Almeida et al. (1997) also did not observe any modification of MDA level in their study but another group reported that increased MDA level is associated with chronic social isolation stress (Zlatkovic et al., 2014). Changes in oxidative stress parameters can vary depending on the model of social isolation, duration of isolation and presence or absence of additional stressors (Gopalakrishnan et al., 2004; Walker et al., 2019).

NO activity in the brain was increased in Group C (Isolated + Distilled water) compared to other groups and this was significant in Group D (Reared + Distilled water). We can infer from our experiments that isolation induced NO activity in rats that were modified by CBD. This may account for why there was no significant difference between Groups A (Isolated + CBD) and B (Reared + CBD). An increase in NO synthetase expression in the cortex of rats exposed to social isolation that correlates with behavioural and histopathological alterations induced by social isolation has been documented by others (Schiavone et al., 2009; Schiavone et al., 2013). CBD has been shown to inhibit NO synthetase and NO in previous studies (Esposito et al., 2006; Costa et al., 2007; Borrelli et al., 2009). However, the effect of CBD on increased NO activity induced by social isolation has not been directly explored before now.

We used α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method to assess the total antioxidant activity of each group (Kedare et al., 2011), we did not observe a significant difference between groups C (Isolated + Distilled water) and D (Reared + Distilled water) suggesting social isolation did not affect the total antioxidant activity in the study. There is no previous work to compare our observation with underlining the need for further research on total antioxidant activity in rat studies of social isolation.

We can conclude from our experiments that social isolation did not affect social affiliation and social novelty in rodents but there is evidence of social isolation-induced anxiety which was not ameliorated by CBD. Social isolation also induced oxidative stress, and this was relieved by CBD.

Limitations of the study

We would have preferred to conduct our experiments using an automated three-chambered apparatus, but this was not available because of financial constraints. Also due to technical difficulties, we measured neurochemical parameters in whole brain tissue rather than in specific brain regions.

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Disclosure statement

Cannabis Science Inc., however, did not contribute towards the development of the protocol, the experiments or the analysis or interpretation of the data.

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