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Synergistic Effects of Pharmacological, Environmental, and Transcranial Direct Current Stimulation Interventions on Depression-Like Behaviours in Rats Under Social and Movement Restriction

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Abstract: *Major depressive disorder (MDD), one of the most prevalent and debilitating mental health conditions, is found to have the highest rates of treatment resistance and recurrence. The current therapeutic approach is primarily based on pharmacological and psychotherapeutic interventions, which fail to achieve complete remission for a significant part of the patients. New interest is emerging in combining pharmacological treatments with neuromodulation and environmental improvements to enhance therapeutic outcomes in this context. This one-year longitudinal study investigated the individual and combined effects of social isolation, chronic restraint stress, sertraline administration, transcranial direct current stimulation (tDCS), and social rehabilitation on depression-like behaviours in male Sprague Dawley rats. Behavioural outcomes were assessed using the Open Field Test, Sucrose Preference Test, Elevated Plus Maze, and Novel Object Recognition Test across ten experimental phases. Social isolation and chronic restraint effectively induced depression-like behaviours, as indicated by reduced exploration and sucrose preference. Sertraline treatment yielded partial improvements in affective and motivational behaviour, though inter-individual variability was notable. The addition of tDCS produced more consistent behavioural benefits and appeared to stabilise recovery trajectories. Social rehabilitation also contributed to behavioural improvement but was influenced by prior treatment history. Depression treatment usually has a high risk of relapse, so continuous interventions and individualised strategies are needed to increase the chance of effective treatment. Our findings support a multimodal approach to depression treatment, where combining pharmacological, neuromodulatory, and environmental interventions offers more significant potential for recovery than single treatments alone. These results underscore the complexity of depressive disorders and the need for long-term, personalised therapeutic strategies.*

Keywords: *major depressive disorder; sertraline; social isolation; chronic restraint stress; tDCS; environmental enrichment; animal model; neuroplasticity; multimodal treatment.*

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1. Introduction

Major depressive disorder (MDD) is the mental health disorder most prevalent globally, affecting over 300 million people and contributing substantially to the global burden of disease (WHO, 2021). Characterised by persistent low mood, anhedonia (loss of interest in previously enjoyable activities), cognitive impairments, and a range of somatic symptoms such as fatigue, appetite changes, and sleep disturbances, MDD often follows a chronic and relapsing course. Despite decades of research and clinical experience, current treatment approaches, primarily conventional pharmacotherapy and psychotherapy, fail to provide lasting relief for a significant portion of patients. Large-scale trials, such as the STAR*D study (sequenced treatment alternatives to relieve depression), revealed that fewer than 50% of patients respond to their first-line antidepressant, and only about 30% achieve complete remission (Rush et al., 2006). Each new failure in treatment attempts decreases the likelihood of remission; therefore, exploring complementary or alternative strategies to enhance therapeutic efficacy represents an important research topic (McIntyre & O'Donovan, 2004).

Traditionally, the monoamine hypothesis, positing a chemical imbalance, most notably a serotonin deficiency, has guided pharmacological treatment strategies for depression. However, recent reviews, such as that by Moncrieff et al. (2022), question the validity of this hypothesis, finding limited evidence that serotonin deficiency is a principal aetiological factor. Despite this, selective serotonin reuptake inhibitors (SSRIs) and related drugs continue to show clinical benefits, likely through mechanisms involving enhanced neuroplasticity and modulation of stress-related neurocircuits rather than merely correcting a neurotransmitter imbalance. This evolving understanding positions MDD as a disorder of impaired neuroplasticity, where chronic stress and environmental adversity may lead to maladaptive alterations in brain structure and function.

In this context, non-invasive neuromodulatory interventions such as transcranial direct current stimulation (tDCS) have gained increasing interest as adjunctive therapeutic modalities in the treatment of depression. tDCS has been shown to influence cortical excitability and synaptic plasticity, potentially reversing maladaptive brain patterns associated with depressive symptomatology. When combined with pharmacological or environmental strategies, tDCS may produce synergistic effects that target multiple domains of dysfunction, including mood, cognition, and motivation (Korai et al., 2021). In our recent review, we emphasised the growing translational potential of transcranial electrical and magnetic stimulation techniques, integrating findings from animal models and clinical studies to support their effectiveness, particularly in treatment-resistant depression (Zamfirache et al., 2025).

Animal models remain indispensable for understanding the neurobiological underpinnings of depression and for testing potential therapeutic approaches. Among these, the chronic mild stress (CMS) paradigm is one of the most widely accepted models. It is capable of inducing core depressive-like behaviours in rodents, such as anhedonia, reduced locomotion, and diminished exploratory behaviour (Willner, 2005). These outcomes closely parallel human depressive symptoms and provide a reliable platform for assessing both behavioural and neurochemical effects of therapeutic interventions.

Furthermore, the COVID-19 pandemic has exacerbated social isolation and physical inactivity, factors independently associated with increased depression risk. This renders exploring such variables in experimental settings particularly timely (Strekalova et al., 2022). Social isolation in rodent models has been shown to induce significant behavioural and neuroendocrine alterations, resembling those seen in human depression, and thus serves as an essential model for studying the impact of environmental deprivation and rehabilitation strategies (Grippo et al., 2007).

This study aims to explore the single versus combined effects of pharmacological (sertraline), environmental (social rehabilitation), and neuromodulatory (tDCS) interventions on depressive phenotypes in rats subjected to social isolation and movement restriction. We hypothesise that a multimodal approach may yield superior outcomes to single interventions by targeting multiple dimensions of depressive pathology—behavioural, cognitive, and neuroplastic.

Given the current limitations of therapeutic methods in treating major depression and considering the complexity of this condition, this study aims to continue the work toward discovering new, more accessible, and effective approaches that are aligned with the personal and neurobiological heterogeneity of depressive disorders in patients.

2. Materials and Methods

2.1. Study Design

This longitudinal study was conducted over one year and included ten sequential experimental stages to evaluate depression-like behaviours and recovery responses in rats. Our protocol was designed to examine the effects of social isolation, chronic stress, pharmacological treatment (sertraline), transcranial direct current stimulation (tDCS), and social rehabilitation. The ten stages of the protocol included: (1) Baseline behavioural assessment; (2) Social isolation; (3) Depression induction via chronic restraint stress; (4) Initiation of sertraline treatment; (5) Exposure to tDCS; (6) Transition to an enriched social rehabilitation environment; (7) Discontinuation of sertraline; (8) Continued behavioural observation; (9) Relapse assessment; (10) Final evaluation (Figure 1).

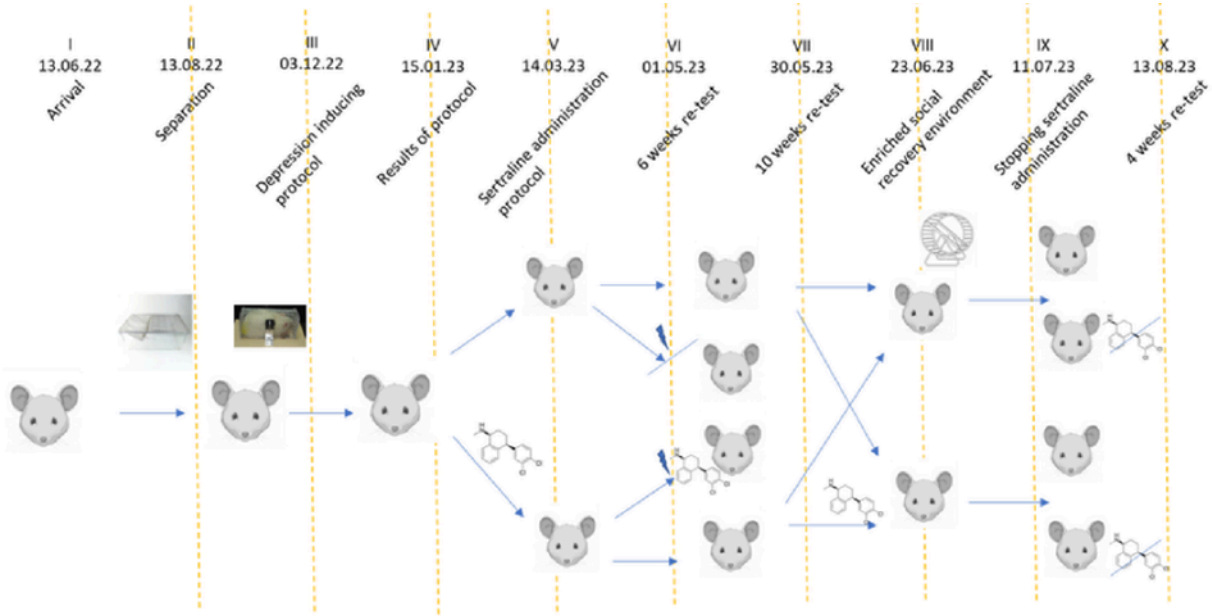

Figure 1. Schematic timeline of the experimental protocol stages conducted over one year. The timeline illustrates the sequential progression of the study, including baseline behavioural assessment, social isolation, chronic restraint stress for depression induction, pharmacological treatment with sertraline, transcranial direct current stimulation (tDCS), environmental enrichment through social rehabilitation, treatment discontinuation, and follow-up assessments. Behavioural tests were administered at key intervals to evaluate affective, cognitive, and motivational changes.

Animals were randomly allocated to different experimental groups based on treatment combinations to assess the effectiveness of individual or combined interventions on behavioural recovery.

2.2. Animals and Housing

Adult male Sprague Dawley rats (N = 10; average weight 150 g) were obtained from the "Cantacuzino" National Institute for Medical-Military Research and Development. Upon arrival, all animals underwent a one-week acclimatisation period. They were housed under temperature-controlled conditions with a 12-hour light/dark cycle. Food and water were provided ad libitum, except during periods involving restraint stress and the sucrose preference test, during which fasting protocols were applied.

All procedures were approved by the University of Bucharest Ethics Committee (Approval No. 14 / 28.04.2021) and carried out per national guidelines provided by the National Sanitary Veterinary and Food Safety Authority regarding the care and use of animals in scientific research.

2.3. Depression Induction

Depressive-like behaviour was induced using the Chronic Restraint Stress (CRS) model. Rats were physically restrained for two hours daily over 2 weeks. This paradigm is widely recognised for its ability to induce behavioural and physiological alterations associated with depressive phenotypes, including hippocampal CA3 pyramidal cell atrophy and elevated corticosterone levels (Magariños et al., 1996). This model simulates prolonged psychosocial stress comparable to that experienced by humans in high-pressure or adverse environments.

2.4. Pharmacological Intervention

Sertraline hydrochloride (Zoloft, Pfizer), a selective serotonin reuptake inhibitor (SSRI), was selected for its proven efficacy and tolerability profile in treating major depressive disorder (Cipriani et al., 2009). Sertraline was administered at a dose of 20 mg/mL, delivered daily via a biscuit to ensure a non-invasive and stress-free administration method, as validated in prior studies (Pawluski et al., 2020).

2.5. Transcranial Direct Current Stimulation (tDCS)

tDCS was applied to modulate cortical excitability and promote neuroplastic recovery. A constant current of 0.5 mA was delivered for 20 minutes per session using a Brain Premier E1 tDCS device connected to a specific build electronic board using silver sponge primers. This device has a direct current stimulation waveform with an intensity from 0.1 to 2.0 mA with an increment of 0.1, with a maximum output of +26V. The duration can be set from 1 to 40 minutes. The cathodal electrode was placed on the left frontal cortex, and the anodal electrode was positioned on the parietal midline of the scalp. Electrode placement and stimulation parameters were based on established protocols for targeting prefrontal circuitry implicated in mood regulation (Fregni et al., 2006; Spezia et al., 2015).

2.6. Social Rehabilitation Environment

In later stages of the protocol, animals were transferred to a custom-designed enriched cage to simulate a supportive, socially stimulating post-treatment environment. The cage featured increased spatial complexity, novel objects, and opportunities for interaction with conspecifics. This environmental enrichment aimed to facilitate behavioural recovery through the stimulation of natural rodent behaviours and neuroplastic mechanisms (Rădulescu et al., 2021).

2.7. Behavioural Assessments

To assess comprehensively the affective, motivational, and cognitive dimensions relevant to depression-like behaviour, four validated behavioural tests were administered at several key points during the experimental protocol. Testing was conducted under consistent lighting conditions (~100 lux) between 10:00 a.m. and 2:00 p.m. to control variability related to circadian rhythms. Before each session, animals were allowed to acclimate to the testing environment. All testing apparatus was cleaned with 70% ethanol between trials to prevent confounding factors from residual scents. This multifaceted behavioural testing strategy allowed for longitudinal monitoring of both the onset and recovery of depression-like phenotypes across different intervention phases.

Open Field Test (OFT): The OFT is used to assess locomotor activity and anxiogenic responses. Each rat was placed in the center of a square arena (60 cm × 60 cm, walls 50 cm high), and behaviour was recorded for 5 minutes using a top-mounted video camera. The arena was virtually divided into a central zone (20 cm × 20 cm) and a peripheral zone. Parameters analysed included total distance traveled (cm), time spent in the central zone (s), and number of entries into

the centre, using an automated behavioural analysis tracking system (ANY-maze software, Dublin, Ireland). Reduced central zone exploration and locomotion are established indicators of anxiety and depression-like behaviour (Seibenhener & Wooten, 2015).

Elevated Plus Maze (EPM): Anxiety-related behaviour was further evaluated using the EPM, a plus-shaped apparatus elevated 50 cm above the cage floor. It consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm), arranged at right angles. Each animal was placed at the center junction in the direction of an open arm and left to explore for 5 minutes. Primary measures included: time spent in open vs. closed arms and the number of entries into each. A reduced proportion of open-arm activity is indicative of anxiety-like behaviour. Video recordings were analysed with manual scoring.

Sucrose Preference Test (SPT): Anhedonia was assessed through the SPT, which evaluates preference for a palatable sucrose solution versus water. Before testing, rats were habituated to a 1% sucrose solution (w/v) for 48 hours. Following a 12-hour water and food deprivation period, animals were given simultaneous access to two pre-weighed bottles, one containing 1% sucrose and the other plain water, for 12 hours. Bottle positions were switched after 6 hours to avoid location bias. A decreased sucrose preference is interpreted as an indicator of anhedonia and impaired reward sensitivity (Willner et al., 1987).

Novel Object Recognition Test (NORT): To assess recognition memory, a cognitive domain often disrupted in depressive states, the NORT was used. Testing was conducted over two days in an open field arena identical in dimensions to the OFT. On day one (habituation), animals explored the empty arena for 10 minutes. On day two (testing), rats were allowed to explore two identical objects (training phase, 5 minutes), followed by a 1-hour retention interval. In the test phase, one of the familiar objects was replaced with a new object that was different in shape, colour, and material. Time spent exploring each object (defined as sniffing or nose-pointing within 2 cm) was recorded.

2.8. Statistical Analysis

Statistical analyses were performed in GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). Data were first screened for normality using the Shapiro–Wilk test to determine the appropriate statistical tests for each dataset. Behavioural parameters, including time spent in the inner zone of the Open Field Test, total distance travelled, sucrose preference, and Elevated Plus Maze arm activity, were treated as continuous variables and analysed accordingly.

For within-group comparisons across time points (e.g., pre- vs. post-intervention), paired two-tailed t-tests were used for normally distributed data. Between-group comparisons (e.g., Control vs. Sertraline or tDCS groups) were conducted using unpaired two-tailed t-tests for independent samples.

The results are presented as mean ± standard deviation (SD), and individual data points were retained and visualised in line and scatter plots to reflect inter-individual variability. Statistical significance was defined as $p < 0.05$, with exact p-values reported throughout the Results section for transparency. For all comparisons, p-values < 0.05 were considered statistically significant, while values between 0.05 and 0.1 were interpreted as statistical trends.

In exploratory analyses, effect sizes (Cohen's d) were calculated for key comparisons to estimate the magnitude of treatment effects, particularly in cases where results approached but did not reach statistical significance.

3. Results

3.1. Impact of Social Isolation on the Onset of Depression-Like Behaviours

To assess the progressive effects of social isolation and movement restriction on depression-like behaviours, we conducted longitudinal behavioural testing using the Open Field Test (OFT) during multiple phases of the experimental protocol. These evaluations aimed to monitor changes in locomotion and anxiety-related behaviours over time, as indicators of emerging depressive symptomatology. The animals were first tested to establish baseline behavioural

parameters under conditions of individual housing, prior to the initiation of the full depression-inducing protocol. Subsequent behavioural assessments were performed at regular intervals throughout the experimental timeline.

As illustrated in Figure 2, the time spent in the inner zone of the Open Field Test exhibits a downward trend over six months, suggesting a progressive increase in depression-like behaviour associated with social isolation ($p = 0.0503$). This result approaches statistical significance, but it does not conclusively demonstrate that early separation into individual cages significantly contributes to the onset of depression.

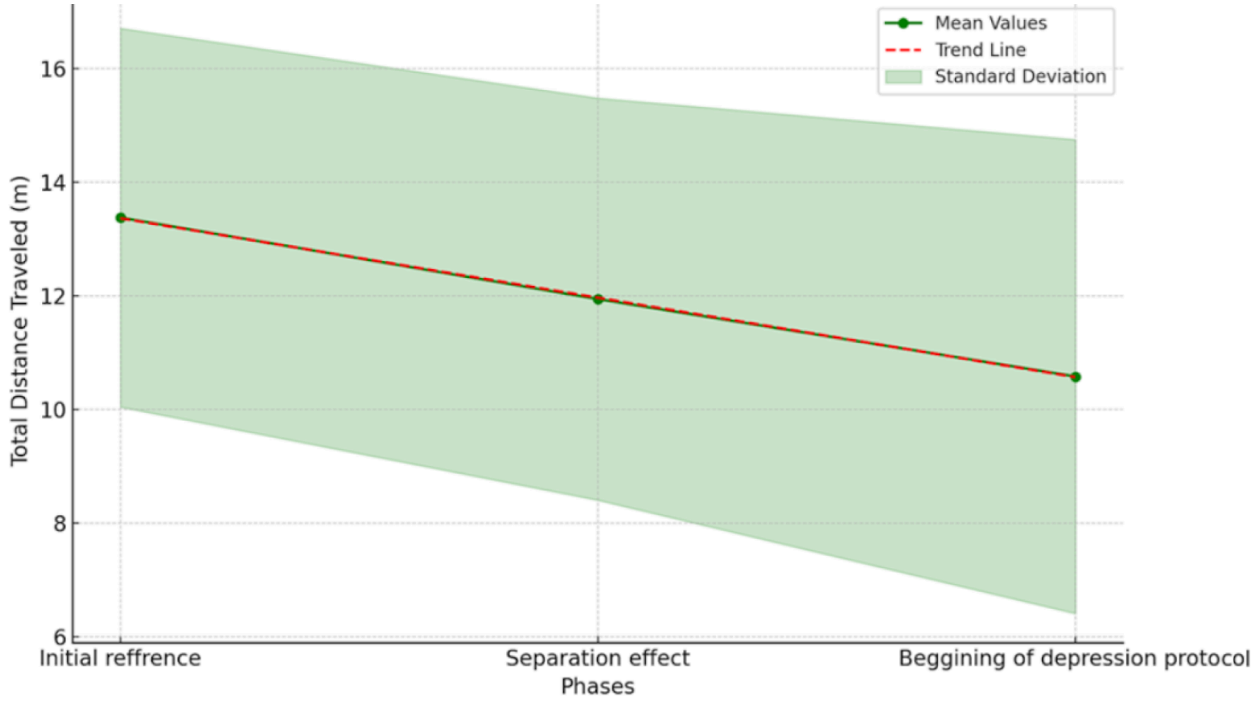

Figure 2. Total distance traveled in the Open Field Test across three experimental phases. The green line represents the mean values of locomotor activity (total distance traveled) during the "Initial Reference," "Separation Effect," and "Beginning of Depression Protocol" phases. The shaded area around the line indicates the standard deviation, reflecting inter-individual variability. The red dashed trend line illustrates a progressive decline in locomotor activity across the three phases

The results of the paired t-tests for total distance travelled indicate a p-value of 0.2477 after two months and a statistically significant p-value of 0.0095 after six months. In light of the above, the reduction in movement observed in the initial as well as in the later phases is unlikely to result from random variation, implying that the depression-inducing protocol had a significant impact on the animals' locomotor function. This is further supported by the line graph in Figure 2, which demonstrates a consistent downward trend in total distance travelled, indicative of reduced physical activity, an established behavioural correlate of depression.

To evaluate changes in anhedonia, paired t-tests were also conducted on sucrose preference data across experimental phases. As shown in Figure 3, the p-values were 0.2707 between the first and second phases, and 0.8771 between the first and last phases, both exceeding the standard threshold for statistical significance ($p < 0.05$). Although these results do not support a statistically significant change in sucrose preference, we can observe a linear trend of increasing preference after two months, followed by a decrease after six months. This pattern may reflect changes in reward sensitivity over time.

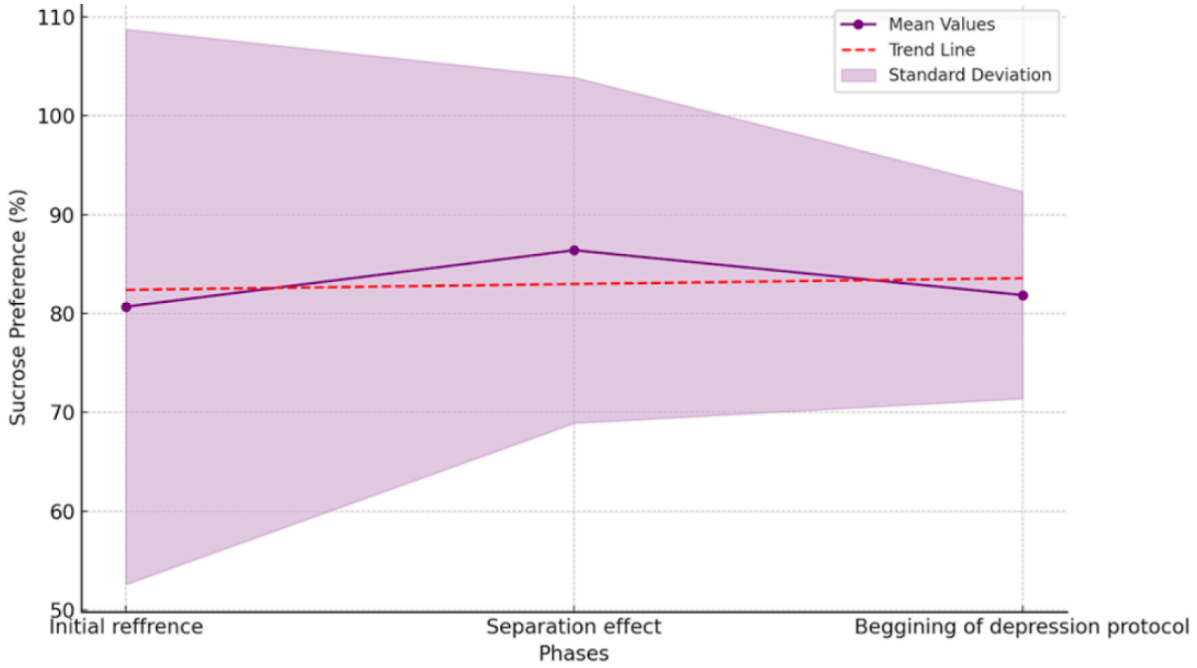

Figure 3. Sucrose preference trends across three experimental phases. The purple line indicates the mean sucrose preference values measured during the "Initial Reference," "Separation Effect," and "Beginning of Depression Protocol" phases. The shaded area surrounding the line represents the standard deviation, illustrating variability within the sample. The red dashed line depicts the overall trend, showing a slight increase from the initial to the separation phase, followed by a decline during the onset of the depression-inducing protocol.

Statistically, the separation did not produce a significant change in the rats' sucrose preference compared to baseline values. This may indicate that, within the context of social isolation, sucrose preference is a less sensitive indicator of depression-like behaviour or that additional confounding factors may have influenced the observed outcomes.

3.2. Tracking Behavioural Decline Under Chronic Restraint

The study progressed with the implementation of the depression-inducing protocol using the chronic movement restriction method (Figure 4). In this next analysis phase, we examined three key time points: the beginning of the depression protocol, after completion, and the onset of sertraline treatment. The analysis focused on alterations in the time spent by the animals exploring the central area of the Open Field arena. A significant difference was observed between the “Start of the Depression Protocol” and “Post-Depression Protocol” phases ($p = 0.0245$), indicating a reduction in centre zone exploratory activity following the chronic stress period. This decline suggests a progression in depression-like behaviour, consistent with reduced exploratory activity and increased anxiety levels.

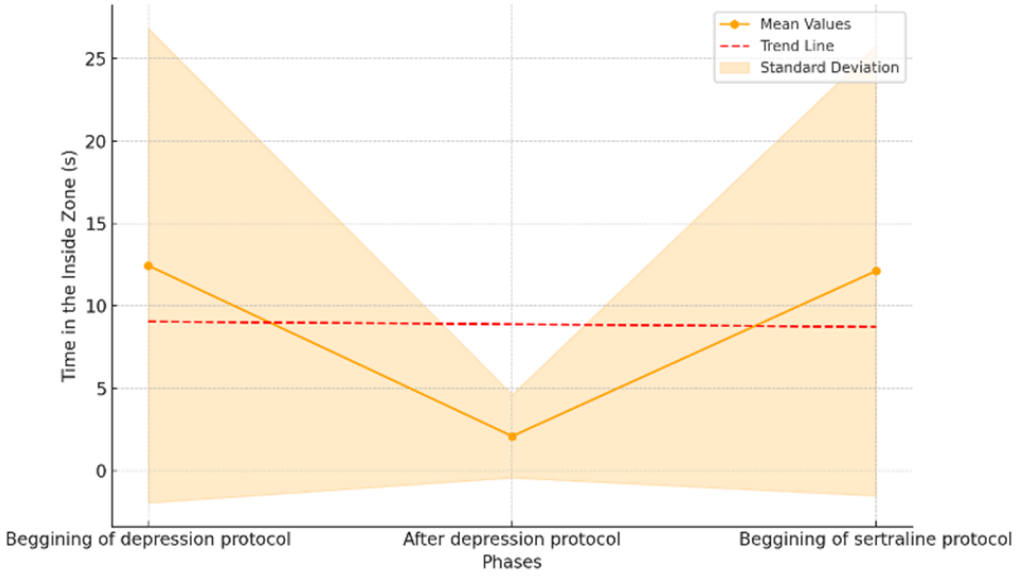

Figure 4. Time spent in the inner zone of the Open Field Test across three key experimental phases. The orange line represents the mean values recorded during the "Beginning of Depression Protocol," "After Depression Protocol," and "Beginning of Sertraline Protocol" phases. The shaded area around the mean illustrates the standard deviation, reflecting variability within the sample. The red dashed trend line indicates a marked decline in center zone activity following the depression protocol, followed by an increase at the onset of sertraline treatment, suggesting a potential behavioural response to pharmacological intervention.

A significant difference was observed between the “After Depression Protocol” and “Beginning of the Sertraline Protocol” phases, with a p-value of 0.0399. This finding indicates a meaningful increase in the time spent in the inner zone of the Open Field Test prior to the initiation of sertraline treatment, suggesting partial behavioural recovery in some animals following the cessation of chronic restraint stress. The chronic stress model produces the expected effects in terms of depression-like behaviours exhibited by subjects. However, variability was evident during the recovery phase, highlighting that the level of resilience of subjects is different and they may have different manifestations of induced depressive states and the mode of recovery from them.

We can observe a downward trend in activity in the central area immediately after the introduction of the depression protocol, which then recovers two months later, before the pharmacological intervention. These observations may suggest that the stress model significantly induces depression-related behaviours, but the mode of manifestation is different across subjects, with some exhibiting spontaneous recovery. Individual variability in these types of experiments is important for both experimental and clinical models of depression.

A substantial increase was observed between the “After Depression Protocol” and “Beginning of Sertraline Protocol” phases ($p = 0.0124$), indicating a notable improvement in overall activity following a one-month interval, mirroring the previously described recovery trend in inner-zone exploration. These results suggest that while depression-like behaviours were present, they did not continue to intensify over time, and some spontaneous behavioural recovery may have occurred before the introduction of pharmacological treatment.

Regarding sucrose preference, no statistically significant difference was found between the “Beginning of Depression Protocol” and “After Depression Protocol” phases ($p = 0.8965$; Figure 5), suggesting that anhedonia, as measured by this test, was not substantially altered during this period.

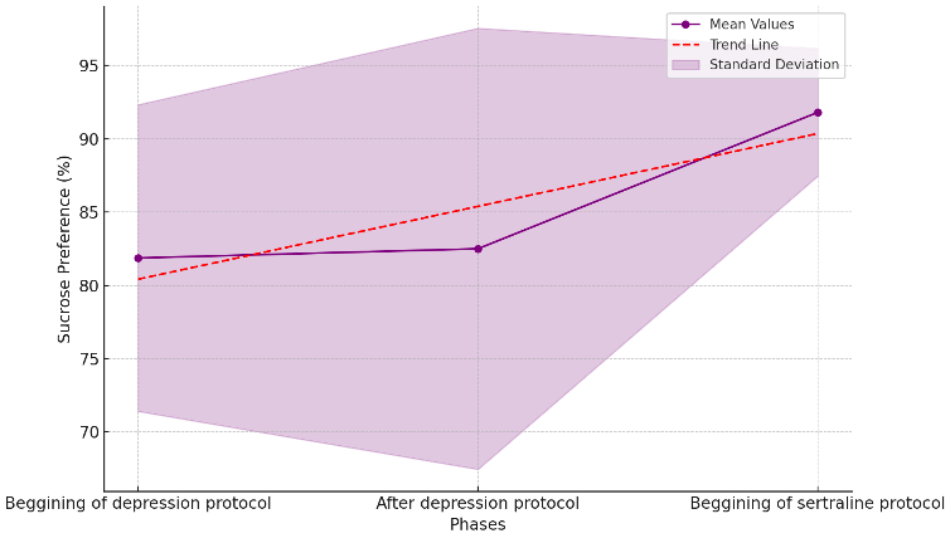

Figure 5. Sucrose preference across three experimental phases. The purple line represents the mean sucrose preference values measured during the "Beginning of Depression Protocol," "After Depression Protocol," and "Beginning of Sertraline Protocol" phases. The shaded area indicates the standard deviation, showing inter-individual variability. The red interrupted trend line presents a slight increase in sucrose preference following the depression phase, with a more notable improvement observed after the beginning of the sertraline treatment, suggesting a potential recovery in hedonic sensitivity.

Consistently, the difference in sucrose preference between the “After Depression Protocol” and “Beginning of the Sertraline Protocol” phases was not statistically significant ($p = 0.0838$). However, it approached the conventional threshold for significance. Although the sucrose preference test revealed a visually apparent trend of improvement in the PE and PEt groups following intervention, these differences did not reach statistical significance ($p > 0.05$). These findings may be interpreted as preliminary and indicative of potential synergistic effects, which warrant confirmation in larger samples.

Statistically, neither the depression protocol, the subsequent two-month interval, nor the initiation of sertraline treatment produced significant changes in sucrose preference within this sample. However, the upward trend may indicate evolving behavioural responses, potentially reflecting increased interest in sweet stimuli. While this could suggest emerging reward-seeking behaviour, possibly even early signs of sugar dependency, it was not pronounced enough to reach statistical significance in the current cohort. To better understand these variables, in-depth studies with larger samples will be needed to help clarify this potential effect.

To prevent misinterpretation, it should be noted that only changes approaching statistical significance (i.e., $0.05 < p < 0.1$) can be cautiously described as trends warranting further exploration. Non-significant results ($p > 0.1$) should not be interpreted as indicative of behavioural improvement without additional converging evidence.

3.3. Behavioural Recovery Following Sertraline Administration

The Control Group exhibited considerable variability in behaviour; however, a general trend showed a decline in total distance travelled by the end of the 6-week phase, followed by a recovery by the 10-week mark (Figure 6). In contrast, the Zoloft Treatment Group experienced a more pronounced decrease in locomotor activity during the “6-week sertraline treatment” phase. The significant initial decrease observed may indicate a deepening of depressive-like behaviour, potentially associated with early adverse effects of sertraline. A significant recovery was subsequently noted, suggesting the onset of sertraline effects.

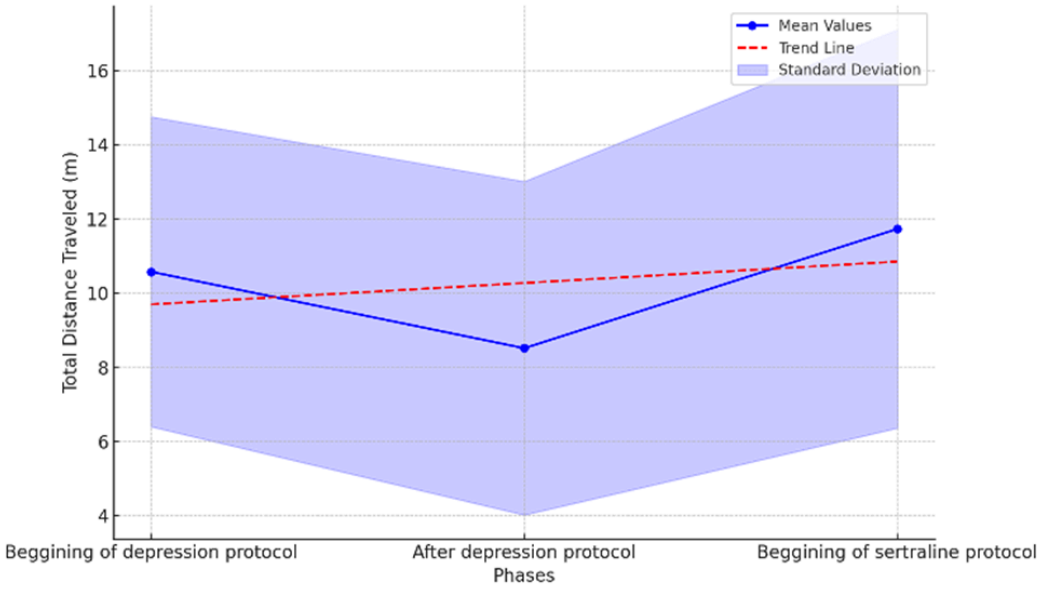

Figure 6. Total distance traveled in the Open Field Test across three experimental phases. The blue line illustrates the mean values of locomotor activity recorded during the "Beginning of Depression Protocol," "After Depression Protocol," and "Beginning of Sertraline Protocol" phases. The shaded area surrounding the line represents the standard deviation, showing the variability within the group. The red dashed trend line indicates the locomotor activity following the depression protocol, with an increase at the onset of sertraline treatment, suggesting a potential reversal of depression-like motor impairments.

Paired t-tests were used to compare the total distance travelled across the sertraline treatment phases for both groups. For the Control Group, comparisons between the “Beginning of Sertraline Protocol” and “6 Weeks of Sertraline Protocol” yielded a p-value of 0.2001, while the comparison between the “6 Weeks” and “10 Weeks” phases resulted in a p-value of 0.5830. Similarly, for the Zoloft Treatment Group, the p-values were 0.1949 and 0.2071 for the same respective comparisons. All values exceeded the conventional significance threshold of 0.05, indicating that the observed changes in total distance travelled were not statistically significant.

Regarding behavioural patterns, the Control Group demonstrated a gradual decline in total distance travelled throughout the sertraline protocol, as evidenced by reduced time spent in the inner zone by the 10-week phase (Figure 7). In contrast, the Zoloft Treatment Group exhibited a sharper decline during the 6-week treatment phase, with a partial rebound at 10 weeks, suggesting an initial exacerbation, followed by incipient behavioural recovery under continued treatment.

The Zoloft Treatment Group exhibited a more pronounced initial decline in sucrose preference, followed by a rebound, potentially reflecting an adaptive response or the gradual emergence of sertraline’s therapeutic effects in mitigating depression-like behaviour.

Moderate but consistent variations were observed in the Control Group across phases, underscoring both the behavioural complexity of depressive states and the heterogeneity in response to stressors. In the Zoloft group, lower variability was observed, up to 10 weeks, suggesting a different way of responding to treatment. In the control group, some rats showed positive behavioural changes, but others were less responsive.

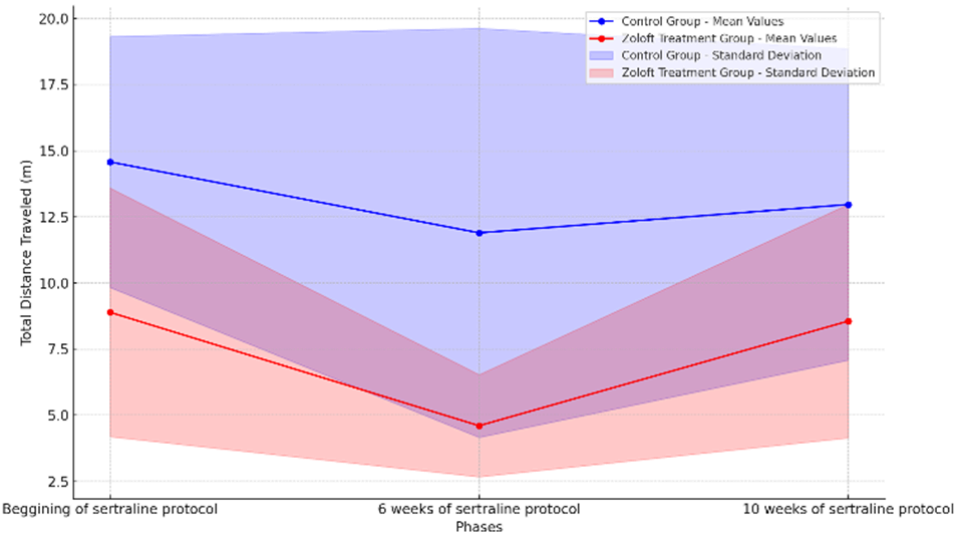

Figure 7. Total distance traveled across experimental phases for the Control and Zoloft Treatment groups. The blue line indicates the mean distance traveled by the Control Group, with the shaded area representing the standard deviation and variability within the group. The red line shows the mean values for the Zoloft Treatment Group, with its corresponding shaded area illustrating within-group variability. This comparison highlights locomotor trends over time between treated and untreated animals.

In the case of sucrose preference (Figure 8), statistical comparisons within the Control Group revealed no statistically significant variation over time. The comparison between the "Beginning of the Sertraline Protocol" and "6 Weeks of the Sertraline Protocol" phases, the t-statistic was 0.7764 ($p = 0.4808$), and between the "6 Weeks" and "10 Weeks" phases, the t-statistic was -1.6688 ($p = 0.1705$). These results suggest that sucrose preference remained relatively stable in the Control Group, with no statistically significant shifts during the observed period.

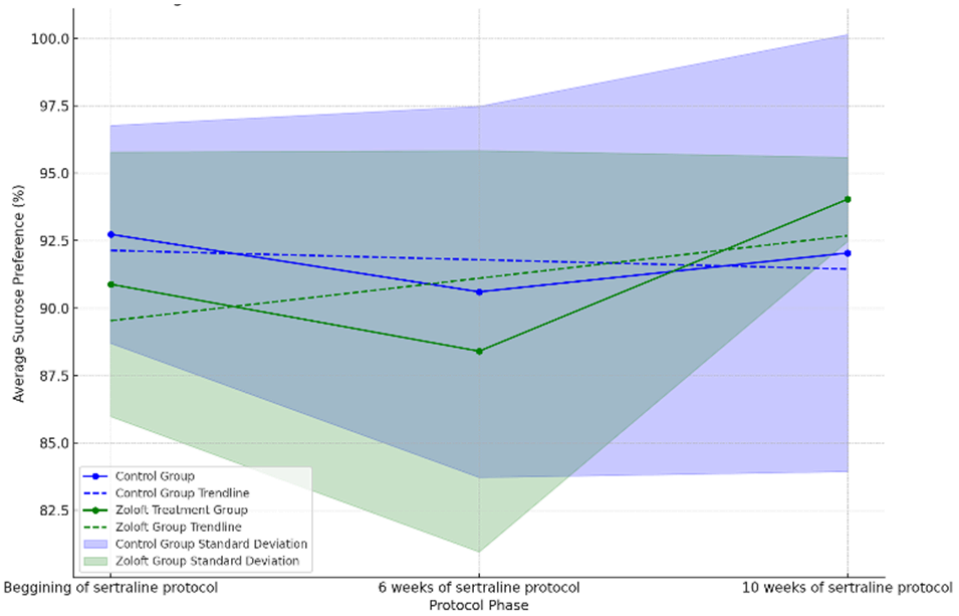

Figure 8. Sucrose preference over time for the Control and Zoloft Treatment groups. The shaded areas represent the standard deviation, illustrating within-group variability across the three phases. The Control Group displays relatively stable variability throughout the timeline, whereas the Zoloft Treatment Group shows greater variability during the "6 Weeks of Sertraline Protocol" phase, suggesting a more heterogeneous behavioural response to the treatment during this period

For the Zoloft Treatment Group, sucrose preference was analysed across time points using paired t-tests. Between the "Beginning of the Sertraline Protocol" and "6 Weeks of the Sertraline Protocol," the t-statistic was 1.0037 with a p-value of 0.3723. Between the "6 Weeks" and "10 Weeks" phases, the t-statistic was -2.1035 with a p-value of 0.1032. As both p-values exceed the conventional threshold of 0.05, they indicate that the differences in sucrose preference across the assessed phases were not statistically significant within the Zoloft Treatment Group.

3.4. Behavioural Changes Following tDCS Stimulation

As illustrated in Figure 9, the tDCS group's sucrose preference started below the overall group average at the onset of the sertraline protocol. However, by the end of the 10 weeks, their preference levels had increased and were close to the group average.

In the case of tDCS, a relatively high and stable sucrose preference was maintained throughout all phases of this protocol. This pattern may signify a constant or improved therapeutic effect, considering sucrose preference as a factor frequently used as a behavioural indicator of anhedonia. The observed trend suggests that tDCS may contribute to maintaining or improving reward sensitivity throughout the treatment.

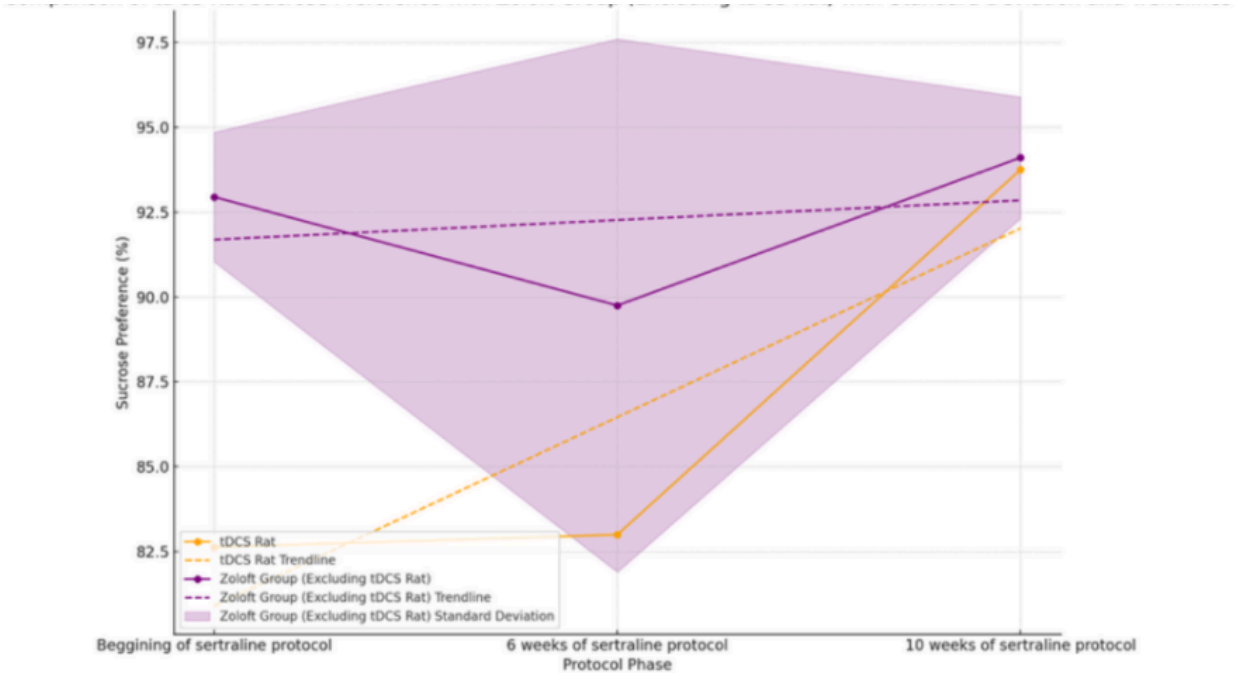

Figure 9. Sucrose preference trends over time for the Control and Zoloft Treatment groups. The shaded areas represent the standard deviation and in-group variability across the three phases. The Control Group shows relatively consistent variability throughout the timeline. The Zoloft group shows increased variability during the "6 Weeks of Sertraline Protocol" phase, indicating a more heterogeneous response to treatment during this period.

Across all protocol phases, the tDCS group consistently demonstrated one of the highest sucrose preference levels within the cohort. This may reflect reduced expression of depressive-like symptoms or enhanced responsiveness to sertraline treatment, particularly in maintaining stable hedonic behaviour.

Notably, sucrose preference remained elevated even after the discontinuation of sertraline, suggesting a potential sustained behavioural effect. This contrasts with the more variable trajectories observed in other groups, some of which showed regression toward baseline levels, indicating reduced treatment durability. At the onset of the sertraline protocol, the sucrose preference difference between the tDCS and the sertraline-only groups was statistically significant. While a noticeable difference remained at the 6-week mark, it did not reach conventional significance

thresholds (e.g., $p < 0.05$). By week 10, the difference had diminished and was no longer statistically meaningful.

This comparative analysis highlights how the addition of tDCS may modulate the trajectory of behavioural recovery, particularly during the early stages of pharmacological treatment.

3.5. Behavioural Outcomes Following Sertraline Discontinuation Without Rehabilitation

The mean time spent in the inner zone of the Open Field Test (Figure 10) indicated no statistically significant differences ($p > 0.05$) between the Zoloft and Control groups at any of the three measured treatment stages. These findings suggest that, within the context of this experimental design, sertraline treatment did not produce a consistently significant effect on anxiety-related behaviour as measured by inner-zone exploration. Figure 10 also illustrates a decreasing trend in centre-zone activity within the Control group over time. Individual variability was more evident initially, indicating that some subjects exhibited greater resistance to the way depressive-like behaviours were initially displayed. Later, this variability decreased, suggesting an alignment of behavioural responses in time.

At the beginning, the Zoloft group had a consistent response after treatment cessation but showed greater inter-group variability later. This behaviour could suggest possible divergent post-treatment trajectories. The control group exhibited differential rates of recovery or behavioural relapse.

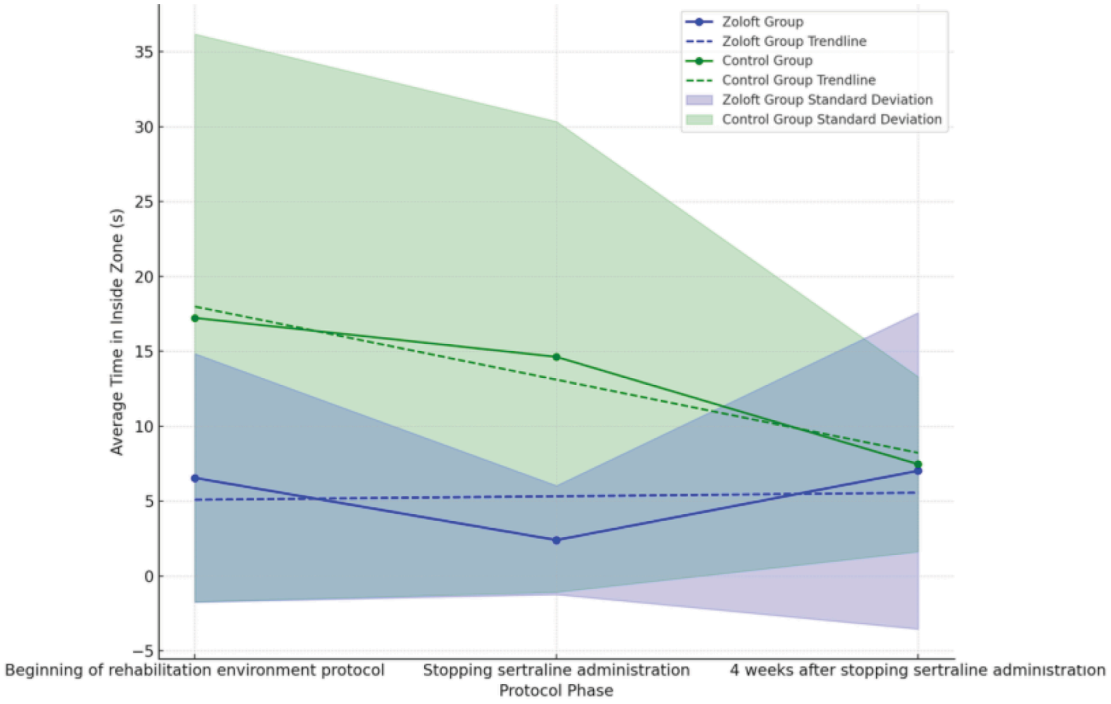

Figure 10. Time spent in the inner zone of the Open Field Test across treatment phases for the Control and Zoloft Treatment groups. The green line represents the mean values for the Control Group, with the shaded area indicating standard deviation and within-group variability. The blue line shows the mean values for the Zoloft Treatment Group, and its surrounding shaded region reflects the corresponding standard deviation. This visualisation highlights trends and variability in anxiety-related behaviour across different stages of treatment.

Figure 11 shows a noticeable difference in mean sucrose preference between the Zoloft and Control groups, with the Zoloft group exhibiting a lower preference. However, this difference is not statistically significant (t-statistic = -2.28, $p = 0.08$), indicating that while there may be a trend toward increased behavioural anhedonia in the Zoloft group at this stage, the evidence does not meet the threshold for significance.

At a later stage, the Zoloft group demonstrated a slightly higher mean sucrose preference than the Control group, suggesting a possible reduction in anhedonia. Nonetheless, this difference

was also not statistically significant (t-statistic = 1.62, p = 0.17), implying that the observed variation could be attributed to random chance rather than a pharmacological influence.

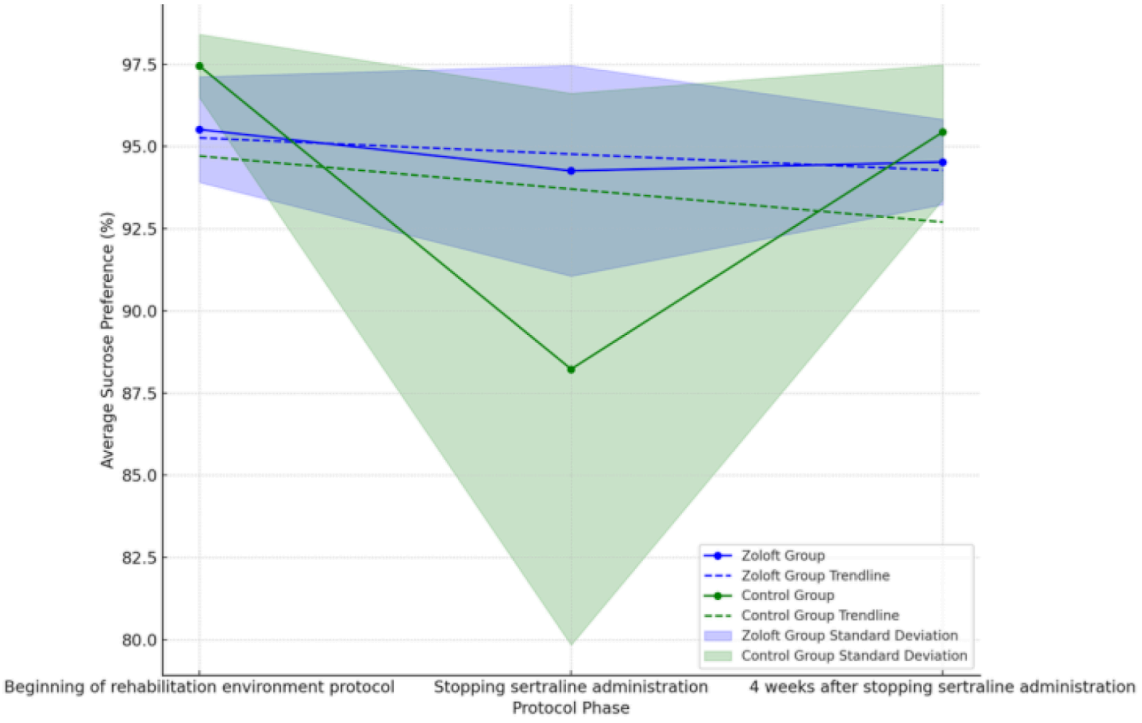

Figure 11. Sucrose preference trends following the discontinuation of sertraline treatment in the Control and Zoloft Treatment groups. The green line represents the mean sucrose preference values for the Control Group, with the shaded green area indicating the standard deviation and reflecting variability within the group. The blue line shows the mean values for the Zoloft Treatment Group, with its shaded region denoting the standard deviation and capturing intra-group variability across the post-treatment phases.

At four weeks following the cessation of sertraline administration, no statistically significant difference was found in the sucrose preference between the Zoloft and Control groups (t-statistic = -0.97, p = 0.30). Both groups exhibited similar mean sucrose preference values, suggesting that the effects of sertraline were no longer distinguishable from the control condition at this stage.

Across all three assessment points, no statistically significant differences in sucrose preference were observed between the Zoloft and Control groups. These findings indicate that sertraline treatment did not produce a measurable reduction in anhedonic behavioural phenotype, as assessed by sucrose preference, relative to the control condition.

Even if not statistically significant, a trend was observed wherein the Zoloft group exhibited a lower sucrose preference at the start of the rehabilitation phase, followed by a modest increase after treatment cessation. This may suggest a transient depressive-like effect that begins to resolve over time; however, additional data and further investigation will be required to validate this pattern.

Significant within-group variability in sucrose preference was observed in both the Zoloft and control groups. This detail highlights individual differences in behavioural response. Future studies with larger sample sizes will be needed to analyse this variability and potential moderating factors involved in order to understand the differential effects of treatment on depression-like behaviours.

3.6. Sertraline Withdrawal Effects in the Rehabilitation Group

At the beginning of the rehabilitation phase, the Zoloft group demonstrated significantly greater depressive-like behaviour compared to the Control group, as reflected by reduced time spent in the inner zone of the Open Field Test (t-statistic = -3.215, $p < 0.05$; Figure 12). We observed a

diminution of the behavioural differences between the two groups, suggesting a potential progressive neurobehavioural recalibration. The pronounced variability in behaviour of the Zoloft group after sertraline discontinuation highlights individual differences in treatment response, underscoring the heterogeneous nature of recovery in depression-like models. This behavioural variability further emphasises subject-specific expression patterns following antidepressant discontinuation in preclinical depression models.

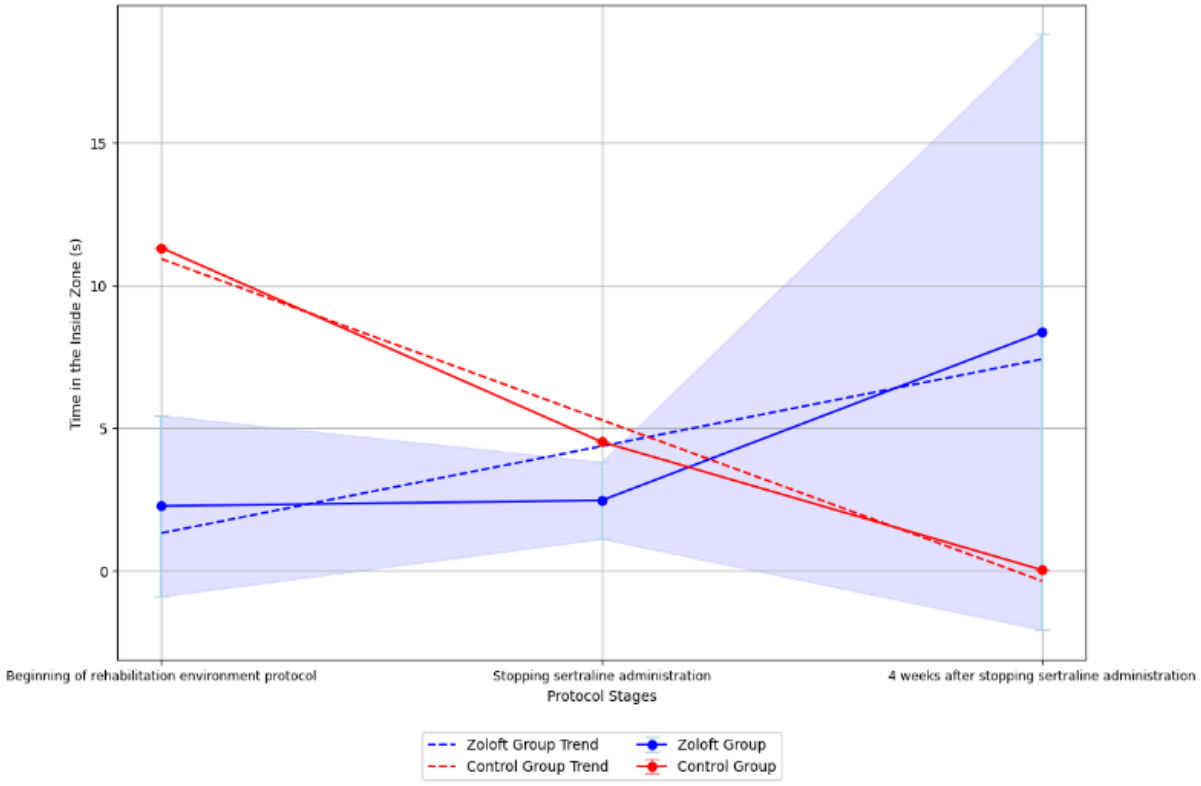

Figure 12. Time spent in the inner zone of the Open Field Test for rats transitioned to the social rehabilitation cage. Shaded areas represent the standard deviation, reflecting within-group variability. The trend lines illustrate the overall behavioural trajectory across the protocol stages for each group, highlighting changes associated with social rehabilitation and treatment conditions.

At the initiation of the rehabilitation protocol, we could not measure a statistically significant difference in sucrose preference between the Zoloft and Control groups (t-statistic = -0.844, $p > 0.05$), as shown in Figure 13. After the discontinuation of sertraline, the Zoloft group showed a higher preference for sucrose compared to the Control group. This could suggest a reduction in depressive-like behaviour. This elevated preference was sustained four weeks post-treatment, indicating a lasting improvement in hedonic function in the Zoloft group compared to controls.

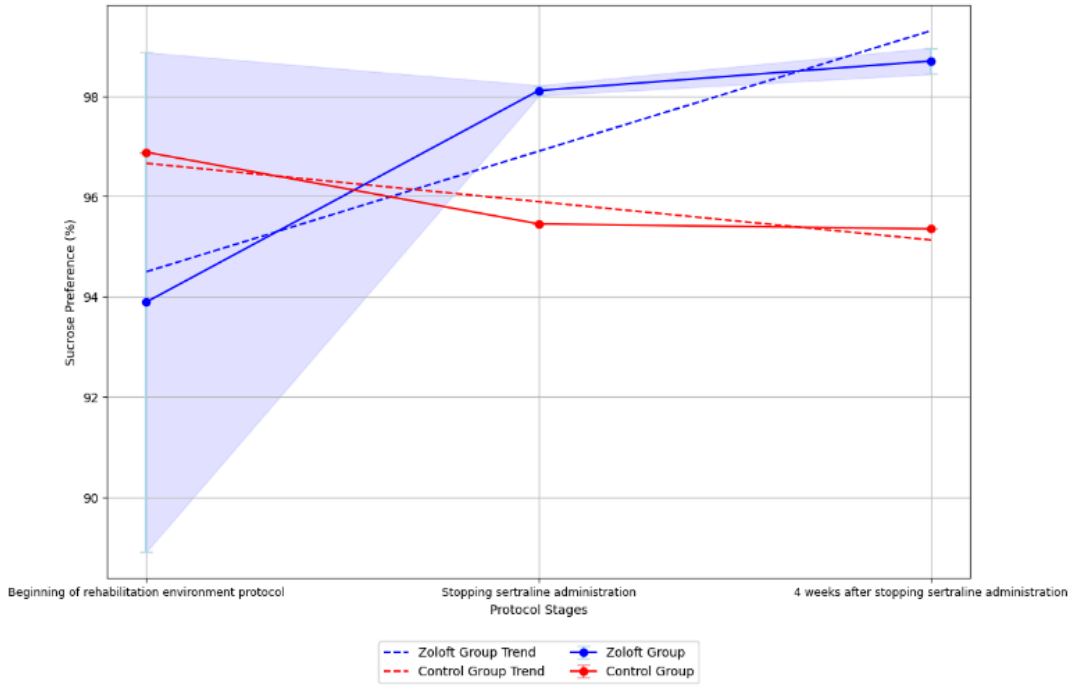

Figure 13. Sucrose preference trends for rats transferred to the social rehabilitation cage. Shaded areas represent the standard deviation, showing variability of each group. The trend lines illustrate the overall progression of sucrose preference across protocol stages, reflecting potential changes in hedonic behaviour in response to environmental and treatment conditions.

3.7. Differences Observed in Behavioural Data

Cohen's d was computed as a normed metric of effect size to assess the magnitude of differences observed in the behavioural data. This metric quantifies the difference between two group means in terms of pooled standard deviation units, providing insight into the practical significance of the findings.

Table 1. Time in the inside zone

Protocol Stage	Zoloft Mean (s)	Control Mean (s)	Zoloft SD	Control SD	Cohen's d	Interpretation
Beginning of rehabilitation environment protocol	2.255	11.3	3.169	N/A	-2.85	Large
Stopping sertraline administration	2.45	4.5	1.345	N/A	-1.53	Large
4 weeks after stopping sertraline administration	8.35	0.01	10.43	N/A	0.80	Large

The Zoloft group exhibited significantly different behaviour compared to the control group across all stages, with robust effect sizes indicating substantial practical differences.

Table 2. Sucrose Preference

Protocol Stage	Zoloft Mean (s)	Control Mean (s)	Zoloft SD	Control SD	Cohen's d	Interpretation
Beginning of rehabilitation environment protocol	93.89	96.88	4.98	N/A	-0.60	Medium
Stopping sertraline administration	98.11	95.45	0.10	N/A	26.60	High
4 weeks after stopping sertraline administration	98.70	95.35	0.26	N/A	12.88	High

Note: Control standard deviations are not applicable (N/A) due to single data points; calculations assume equal variances.

4. Discussions

This study explored the complex interplay between social isolation, chronic movement restriction, pharmacological intervention (sertraline), environmental enrichment, and neuromodulation (tDCS) in modulating depression-like behaviours in a rat model. Over a year, this long-term protocol allowed for the assessment of both the development and progression of depressive phenotypes and the efficacy of various single and combined interventions.

Initial phases demonstrated that social isolation alone, particularly over extended periods, contributed to decreased locomotor activity and reduced time spent in the inner zone of the open field, both indicative of elevated anxiety and depression-like behaviour (Strekalova et al., 2022). While the sucrose preference test did not yield statistically significant changes during this phase, the decline in behavioural patterns is consistent with prior literature suggesting that chronic stress and isolation can gradually alter motivation and reward sensitivity (Willner et al., 1987; Loas & Boyer, 1996).

The chronic restraint stress model further accentuated these depression-like symptoms, including reduced mobility and exploratory behaviour, as expected based on its established efficacy in inducing hippocampal atrophy and behavioural despair (Magariños et al., 1996). These behavioural changes parallel core symptoms of MDD in humans and reinforce the validity of CRS as a translational model (Wang et al., 2017; Planchez, Surget, & Belzung, 2019). Importantly, spontaneous recovery was observed in some individuals following stress cessation, aligning with findings from resilience research that suggests endogenous recovery pathways may buffer against long-term psychopathology (Russo et al., 2012).

Sertraline treatment led to moderate improvements in behavioural measures. An increase in time spent in the centre of the open field and total distance travelled was observed after several weeks of administration, reflecting enhanced affective and motor activity. However, the effects were not uniformly distributed across subjects, suggesting inter-individual variability in treatment response, a phenomenon well-documented in clinical populations (Rush et al., 2006; McIntyre & O'Donovan, 2004). Although not statistically significant, the modest changes in sucrose preference are consistent with other animal studies indicating that SSRIs may take several weeks to produce robust changes in reward sensitivity and hedonic tone (Gemmel et al., 2016; Belzung & Lemoine, 2011).

The addition of the tDCS social buffering" hypothesis, which posits that enriched social contexts mitigate the neurobehavioural impacts of stress and promote recovery through oxytocinergic and dopaminergic neuromodulatory cascades (Hostinar, Sullivan, & Gunnar, 2014; Kikusui, Winslow, & Mori, 2006).

A sudden stop of sertraline administration led to divergent outcomes. Some animals maintained improvements, while others exhibited relapse-like behaviours. This outcome suggests the need for post-treatment support systems in translational applications. Discontinuation syndromes and relapse are well-documented challenges in SSRI-treated populat stabilised and potentially enhanced behavioural outcomes. Rats receiving both sertraline and tDCS exhibited higher and more consistent sucrose preference scores and center exploration behaviour, particularly in later phases of the protocol. These findings align with clinical and preclinical evidence suggesting that tDCS facilitates synaptic plasticity and functional connectivity in prefrontal-limbic circuits, contributing to affective modulation and cognitive restoration (Fregni et al., 2006; Nitsche & Paulus, 2009; Kuo, Paulus, & Nitsche, 2014).

Environmental enrichment through social rehabilitation also influenced recovery, though outcomes varied. While some rats demonstrated stable improvements after being moved to socially enriched environments post-treatment, others did not, suggesting that environmental interventions may be most effective when personalised or paired with other therapies (Strekalova et al., 2022; Rădulescu et al., 2021). These findings resonate with the "ions (Fava et al., 1997), and this preclinical model mirrors such dynamics, reinforcing the need for comprehensive longitudinal management strategies.

Our results reinforce the growing view that MDD is a multifactorial disorder best addressed through integrative treatment paradigms considering behavioural, biological, and environmental factors (Moncrieff et al., 2022; Belzung, Willner, & Philippot, 2015). The high variability observed in both behavioural trends and treatment outcomes underscores the limitations of single-target therapies and the importance of tailoring interventions to individual profiles. This perspective aligns with precision psychiatry approaches advocating for stratified treatment protocols based on neurobiological, genetic, and behavioural biomarkers (Insel, 2014).

This research highlights the translational importance of combining pharmacological, neuromodulatory, and environmental therapies to treat this complex affective disorder. Future studies should explore mechanisms underlying resilience, potential sex differences, and the long-term neurobiological consequences of each intervention. It would also be beneficial to systematically integrate biomarkers (e.g., BDNF levels, corticosterone, neuroinflammation) to correlate behavioural outcomes with underlying physiological processes, as hypothesised mechanisms.

While the observed behavioural improvements in sucrose preference and time spent in the inside zone following tDCS and sertraline interventions suggest a potential therapeutic effect, the underlying neurobiological mechanisms remain unelucidated. Previous literature has associated such behavioural changes with processes involving neuroplasticity, HPA axis regulation, and reductions in neuroinflammatory signalling. However, our current study did not include molecular, hormonal, or histological assessments (e.g., BDNF expression, hippocampal volumetry, or corticosterone levels), and therefore, we cannot confirm whether these pathways were directly involved.

The Elevated Maze and Novel Object Recognition Tests did not show any significant results over a longer period, showing a potential limitation of these methods in re-testing several times during different treatment phases for long periods. While some behavioural measures showed numerical changes following intervention, many did not reach statistical significance.

Importantly, we have refrained from interpreting non-significant results as evidence of therapeutic efficacy. Where effect sizes were calculated, they were used solely to contextualise magnitude and not as evidence of effect in the absence of statistical significance.

We emphasise the exploratory nature of these findings and recommend caution in drawing firm conclusions until replicated in studies with greater statistical power.

5. Conclusions

This study demonstrates that a multimodal approach by combining pharmacological treatment with sertraline, non-invasive neuromodulation via tDCS, and environmental enrichment through social rehabilitation can produce more robust and sustained outcomes in depression-like behaviours in rats than monotherapies in isolation. Each modality contributed distinct effects on affective, cognitive, and motivational outcomes, underscoring the multifaceted nature of depressive disorders and the necessity for integrative treatment strategies.

Sertraline treatment showed modest but beneficial effects, particularly in specific exploratory behaviour and sucrose preference, and its effects were inconsistent across individuals. Adjunct tDCS appeared to stabilise the results, indicating a synergistic neuromodulatory effect in the treatment of depression.

The improved recovery environment had mixed but generally positive effects, emphasising the importance of external factors in post-depression recovery and relapse prevention.

The overall variability observed during the study, in the behavioural responses during and after treatment, highlights the limitations of standardised, one-size-fits-all interventions. It reinforces the need for personalised, adaptive therapeutic frameworks in preclinical and clinical settings. These findings contribute to the ongoing paradigm shift toward precision psychiatry and support further exploration of combined interventions that target multiple neurobiological systems implicated in mood regulation.

6. Limitation

A key limitation of this study is the small sample size, which reduces statistical power and may increase the risk of both Type I and Type II errors. While some trends in behaviour (e.g., an apparent increase in sucrose preference) suggest beneficial effects of combined interventions, these findings should be interpreted with caution. Further studies with larger sample sizes are essential to validate these initial observations and confirm the synergistic potential of multimodal treatments. Future research should continue the work needed to validate these results in larger cohorts, including physiological biomarkers, and evaluate the long-term durability of combined interventions. Such efforts will be essential in translating multimodal therapeutic strategies from animal models to clinical practice in the treatment of major depressive disorder. Another important limitation of this study is the absence of molecular or histological analyses (e.g., BDNF, cytokines, corticosterone levels), which prevents definitive conclusions about the underlying neurobiological mechanisms. Future studies should include such assays to validate the role of neuroplasticity and HPA axis modulation in mediating the observed behavioural effects.

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