Title: The Mitochondrial Toxicity Induced by 3-Nitropropionic Acid Enhanced Susceptibility to Defective Social Behaviors in Male Wistar Rats: Possible Role of Glucocorticoid Receptor and FKBP5

Running Title: 3-NP and Social Interaction

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Abstract

The impaired mitochondrial function in neurons is a core abnormality in many medical conditions. Behavioral changes are the key aspects that emerge under these conditions. In the current study, we investigated whether social interactions are influenced by 3-nitropropionic acid (3-NP)-induced mitochondrial failure. We also assessed changes in glucocorticoid receptor and FKBP5 protein levels, cytochrome contents, and monoamine oxidase A and B activities in the striatum, hippocampus, and prefrontal cortex of the subjects. Adult male Wistar rats were treated with 3-NP. The social and non-social behaviors of 3-NP-treated rats were investigated. Different dissected brain regions were considered in terms of glucocorticoid receptor and FKBP5 protein levels, cytochrome contents, and monoamine oxidase A and B activities. We found a significantly decreased duration of social behaviors along with impaired non-social behavioral tests in the striatum, hippocampus, and prefrontal cortex. We detected a decreasing trend in the levels of glucocorticoid receptor and FKBP5 protein. Moreover, cytochrome contents and monoamine oxidase A and B activities decreased in the dissected brain regions. Impaired social/non-social behaviors along with decreased levels of investigated molecular variables in the aforementioned regions after 3-NP treatment might point to processes connecting mitochondrial failure to behavioral impairment, particularly social type.

Keywords: 3-NP, Glucocorticoid receptor, FKBP5, Cytochrome, Monoamine oxidase, Social behavior

Introduction

Normal mitochondrial function is a pivotal player in maintaining the cellular physiological state through mechanisms such as providing energy and buffering cytosolic Ca^{2+} concentration (Rizzuto et al., 2012). There are vast numbers of biochemical reactions that depend on or can be modulated by the level of cellular adenosine triphosphate (ATP) and Ca^{2+}. Therefore, the mitochondrial function is a hub that indirectly sends modulatory signals to the cellular machinery. The importance of mitochondria in neurons is even more prominent because of their high energy demands (Hyder et al., 2013). Mitochondrial failure in neurons can affect their excitability, neurotransmitter release, and eventually their viability (Kann & Kovacs, 2007).

In several medical settings, decreased mitochondrial function in neurons is the main challenge. It may be acute and have an extrinsic origin, like when the oxygen and/or glucose supply is disturbed in the brain (e.g., ischemic and hemorrhagic stroke, perinatal hypoxia, and prolonged cardiopulmonary resuscitation), or may be chronic and intrinsic, like when the mitochondrial energetic capacity of neurons is reduced (e.g., aging and neurodegenerative diseases). These functional losses decrease the level of available ATP, increase reactive oxygen species (ROS), and disturb Ca^{2+} hemostasis (Yin et al., 2014). The consequences of these turbulent events in the brain can be observed at the cellular and behavioral levels.

One of the obvious features that can be significantly influenced by altered mitochondrial function is behavioral change (social or non-social type). Significantly altered behaviors have been reported in several models with impaired mitochondrial function (Kupsch et al., 2014; Pacelli et al., 2010; Watson et al., 2014). One model characterized by impaired mitochondrial function is the 3-nitropropionic acid (3-NP) model, which is widely used to imitate Huntington's disease (HD) symptoms (Borlongan et al., 1997). Although several studies have investigated different

behavioral changes, including altered locomotor activity and memory, in the context of 3-NP exposure (Dhir et al., 2008; Jain & Gangshettiwar, 2014; Puneet Kumar et al., 2010; Thangarajan et al., 2014), evaluation of social interaction and other non-social behaviors is quite limited.

The activity of the hypothalamic-pituitary-adrenal (HPA) axis is a modulator of behavior (Iniguez et al., 2014; Packard et al., 2016). For example, overactivity of the HPA axis has adverse behavioral consequences like anxiety (Faravelli et al., 2012), depression (Pariante & Lightman, 2008), and impaired social behaviors (Bagosi et al., 2017). The glucocorticoid receptor (GR) in the brain participates in the feedback loop and controls HPA axis activity. Alongside GR, FK506 binding protein 5 (FKBP5) is a cellular protein that modulates GR function (Zannas et al., 2016). Despite these roles, it is unclear how these proteins respond to mitochondrial failure and complex II inhibition in particular. Measuring the content of mitochondrial cytochrome may also help to gain a greater understanding of the processes after complex II inhibition in the electron transport chain.

Monoamine oxidase (MAO) enzymatic activity is another possible agent that can have significant impacts on controlling behavior and contribute to the pathogenesis of a wide range of mental disorders and neurodegenerative diseases, from antisocial personality disorder to Parkinson's disease (Bortolato and Shih 2011). Sociobehavioral disorders due to disruption of mitochondrial processes can be related to the alteration of MAO activity (Bortolato et al., 2013).

In the current study, we investigated whether social or non-social behaviors are influenced by mitochondrial complex II inhibition. To better understand the underlying mechanisms, we also measured the levels of GR, FKBP5 protein, cytochrome contents, and monoamine oxidase A and B (MAO-A and MAO-B) activities in the striatum (ST), hippocampus (HIP), and prefrontal cortex

(PFC) of rats after 3-NP treatment. The findings of this study suggest a relationship between mitochondria and social behavior.

Materials and Methods

Animals

Adult male Wistar rats (220-240 gr) were obtained from the Laboratory Animal Center of Pasteur Institute, Tehran, Iran. Rats were kept under controlled environmental conditions of constant temperature (23 ± 2 °C) and a 12 h light/12 h dark cycle. The animals had access to food and water ad libitum. All animal care and experimental procedures were performed in accordance with the guidelines of the National Health Association and had the approval of the Shahid Beheshti University of Medical Sciences Ethics Committee and Neuroscience Research Center Ethics Board (IR.SBMU.PHNS.REC.1399.167).

Drug Administration

The animals were divided into two groups: control (which received the vehicle daily) and treatment. 3-NP (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in saline. The rats in the treated group received 3-NP treatment (20 mg/kg/day, intraperitoneally (ip)) for five consecutive days (n=8-10/group), as it was approved for the HD model.

Behavioral tests

Before conducting the social interaction test, the animals were habituated in a row in an open field arena (OF). On the test day, the rats were separately segregated into small cages in the test room for three hours. After that, two unfamiliar rats were placed in opposite corners of the OF arena, and their activities were videotaped for 10 minutes. The test was recorded using a video camera suspended two meters above the testing apparatus. Immediately after behavioral testing, the

animals were returned to their cages and OF was completely wiped with 70% ethanol and tissue paper to reduce any lingering olfactory cues.

The observed behaviors were divided into the following two categories: 1. Social behaviors: including social nose contact (advertent nose-to-body contact with the other rodent), genital investigation (sniffing or assessing the anogenital region), and play behaviors consisting of following (chasing the other rodent within a tail length distance), wrestling (harsh and tumble play), and pouncing (one rodent nosing or rubbing the nape of the other one) (Mikulecká et al., 2014); 2. Non-social behaviors (Manfré et al., 2018), including exploring (walking around the field and sniffing the walls and floor), self-grooming (any grooming behavior that a rodent performs in a normal sequential pattern to clean its own body and fur) (Silverman et al., 2010), rearing (the total time that the rodent temporarily stands on its hind legs with the intention exploring) (Valvassori et al., 2017), solitary behavior (activities that are not aimed toward another rat), and immobility (del Angel Ortiz et al., 2016; Lorbach et al., 2018). Behaviors were blindly scored and assessed by two independent observers for each animal. The duration of time spent by two rats engaging in social behaviors, including play behavior, genital investigation, and social nose contacting was also calculated to assess their total social behavior (Lech et al., 2021).

Tissue preparation

After assessment of behavioral tests, rats were euthanized by CO2 asphyxiation and decapitated. The brains were removed and the ST, HIP, and PFC were dissected on ice and frozen in liquid nitrogen, and then stored at -80 ^{o}C for further molecular analysis.

Western Blotting

Different dissected brain regions (ST, HIP, and PFC), based on the Paxinos and Watson's stereotaxic atlas, were homogenized in a lysis buffer (Roche, Penzberg, Germany). The Bradford

colorimetric method was used to determine protein concentration (Bradford, 1976). Sixty micrograms of total protein were loaded on sodium dodecyl sulfate (SDS)-polyacrylamide gels and were separated by electrophoresis. Proteins were electrotransferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were blocked and incubated with primary antibody overnight at 4°C, and then with secondary antibody for 90 minutes. Immunoreactive polypeptides were revealed by enhanced chemiluminescence reagents. The protein bands were analyzed by Image J software and normalized to Beta-actin as a housekeeping protein. The following primary antibodies were used: GR, FKBP5, Beta-actin (1:1000, Cell Signaling Technology, Beverly, MA, USA), and secondary HRP-conjugated anti-rabbit antibodies (1:3000, Cell Signaling Technology, Beverly, MA, USA).

Cytochrome Measurements

The method of Williams (1964) (Williams Jr, 1964) was applied to determine the content of various cytochromes in isolated mitochondria (Clark & Nicklas, 1970). A double-beam scanning spectrophotometer was used to get the cytochrome spectra of the mitochondria. Small amounts of sodium dithionate and hydrogen peroxide were added to obtain the reduced minus-oxidized spectra (Jones & Poole, 1985; Kumar et al., 2008; Mehrotra et al., 2015).

Monoamine oxidase A and B activities

To estimate MAO-A activity, the sample was mixed with a buffer containing sodium phosphate buffer and 5-hydroxytryptamine (4 mM). The change in absorbance was recorded at a wavelength of 280 nm against the blank. To estimate the MAO-B activity, the sample was mixed with sodium phosphate buffer and benzylamine (100 mM). Then, absorbance was recorded at a wavelength of 249.5 nm against the blank (Dhingra & Goyal, 2008; Saleem et al., 2023).

Data Analyses

All the results were expressed as mean ± SEM. The normal distribution of data was checked using the Kolmogorov–Smirnov test. The data were analyzed using unpaired t-tests. The data were analyzed using unpaired t-tests. The statistical analyses were performed using GraphPad Prism 9.5.1 software (San Diego, CA). Statistical significance was accepted at p-value < 0.05.

Results

Social interaction test analysis

Social behaviors: The behavioral test yielded that 3-NP injected rats had a significantly lower duration of play behavior (Figure 1A) and genital investigation (Figure 1B) than those in the control group with the mean±SEM of 12.37±0.46 seconds vs 26.87±2.70 seconds for play behavior (P value= 0.0001, t value= 5.290) and 4.37±0.75 vs 20.62±3.59 seconds for genital investigation (P value= 0.0006, t value= 4.423). The duration of social nose contact in 3-NP-treated animals was lower than the control rats, however, this difference was not statistically significant (24.37±3.49 seconds vs 28.87±3.69 seconds, respectively, P value= 0.3909, t value= 0.885, Figure 1C). Total time spent on social behavior in the 3-NP group (41.12±3.80 seconds) decreased significantly in comparison with the control (76.37±6.73 seconds, P value= 0.0005, t value= 4.553, Figure 1D).

Non-social behaviors: There was a significant decrease in the rearing (P value= 0.0001, t value= 5.057, Figure 2A) and solitary behavior (P value= 0.0006, t value= 4.271, Figure 2D) time in the exposed rats in comparison with the control ones (28.00±3.52 seconds vs 65.12±6.96 seconds for rearing and 282.0±13.14 seconds vs 368.50±15.59 seconds for solitary behavior). Although self-grooming (P value= 0.154, t value= 1.495, Figure 2B) and exploring time (P value= 0.143, t value= 1.540, Figure 2C) also showed decreasing trends, they did not differ significantly between the

groups (46.30±6.09 seconds vs 60.12±6.98 seconds for self-grooming and 207.70±12.50 seconds vs 243.25±20.63 seconds for exploring). Additionally, significantly longer periods of immobility were seen in the injected group when compared to the control (177.77±25.62 seconds vs 12.75±7.80 seconds, respectively, P value<0.0001, t value= 5.842, Figure 2E).

Molecular analysis

GR levels decreased. In the ST, the GR level was significantly lower than in the control animals (P value< 0.0001, t value= 8.922, Figure 3A). The hippocampal GR level in the injected rats was also considerably lower than the control (P value<0.0001, t value= 12.82, Figure 3B). However, the GR level in the PFC did not alter following the treatment (P value= 1.102, t value= 1.558, Figure 3C).

FKBP5 levels declined. The striatal FKBP5 level in 3-NP exposed rats was significantly lower than the control ones (P value= 0.024, t value= 2.651, Figure 3D). A considerable decrease was also observed in the level of the hippocampal FKBP5 compared to the control (P value< 0.0001, t value= 28.52, Figure 3E). Moreover, a noticeable decrease was detected in the FKBP5 level of PFC in comparison with the non-treated rats (P value= 0.0009, t value= 4.652, Figure 3F).

Cytochrome contents decreased. The quantity of striatal cytochrome aa_3 (Cyt aa_3), b (Cyt b), c (Cyt c), and c_1 (Cyt c_1) decreased significantly compared to the control group with the P values< 0.0001, and t values were 8.526, 10.58, 7.37, and 7.642 respectively (Figure 4A). In the HIP, 3-NP group had meaningfully decreased content of Cyt aa_3 (P value< 0.0001, t value=6.967), Cyt b (P value= 0.0006, t value= 4.614), Cyt c (P value= 0.004, t value= 3.503), and Cyt c_1 (P value= 0.003, t value= 3.597) in comparison with the non-treated animals (Figure 4B). According to the cytochrome contents of PFC, the 3-NP treatment decreased the levels of Cyt aa_3 (P value= 0.019, t value= 2.793) and Cyt b (P value= 0.024, t value= 2.656). However, the levels of Cyt c and Cyt

c_1 did not alter following the 3-NP treatment (P value= 0.648, t value= 0.470, and P value= 0.361, t value= 0.955, respectively, Figure 4C) compared to the control group.

MAO-A and MAO-B activities decreased. In comparison with the control group, the MAO-A activity was significantly lower in the 3-NP-treated animals in STR (50.40% of control, P value= 0.001, t value= 3.994), HIP (80.75% of control, P value= 0.006, t value= 3.262), and PFC (81.45% of control, P value= 0.023, t value= 2.556). Similarly, MAO-B activity in the 3-NP group showed a significant decline in STR (P value= 0.001, t value= 3.939), HIP (P value= 0.007, t value= 3.158), and PFC (P value= 0.043, t value= 2.242) compared to the control groups (39.84, 78.20, and 81.76 % of control, respectively; Figures 5A- 5F).

Discussion

We found that 3-NP treatment impaired the social behavior index. Certain non-social behaviors have also been impacted. It also reduced the levels of GR, FKBP5 protein, cytochrome contents, and MAO-A and MAO-B activities in the studied brain regions.

There is a broad spectrum of medical conditions with mitochondrial failure that can impair social behavior, including cerebral hypoperfusion (Lee et al., 2015), hypoxia (Chauhan et al., 2022), ischemia (Girard et al., 2014), Alzheimer's disease (Filali et al., 2011), or autism spectrum disorders (Frye, 2020; Rossignol & Frye, 2012). There are indeed numerous animal studies that investigated affective symptoms in these disorders (Prasad & Hung, 2020; Ruan & Yao, 2020; Southwell et al., 2018). The literature suggests that the inhibition of complex I in adulthood leads to reduced social interaction (Madiha & Haider, 2019; Siena et al., 2021). However, the role of mitochondrial complex II in regulating social behaviors remains unknown. It subsequently inspired the concept of designing this study.

There is also cumulative evidence showing the participation of our studied brain regions in the processing of social behaviors. For example, social interaction in rats has been reported to be associated with increased c-Fos expression as a marker of cellular activity in PFC and ST (van Kerkhof et al., 2014). The inactivation of different subregions of PFC led to impaired play behavior (van Kerkhof et al., 2013). Also, impaired glutamatergic signaling in the ST and HIP modulated social behavior (Finlay et al., 2015; van Kerkhof et al., 2013). The structural features like dendritic length and density of dendritic spines in PFC and HIP (Silva-Gomez et al., 2003) and the oxidative state in PFC and ST (Moller et al., 2011) affect this behavior.

We found that 3-NP significantly impaired social interaction. It reduced the duration of play behavior, genital investigation, and the total time spent on social behaviors. These findings are in line with the aforementioned conditions of mitochondrial failure. 3-NP is also used to replicate HD symptoms in rats. Similar to our observations, HD transgenic animals also show reduced levels of social interaction (Manfré et al., 2018; Wood & Morton, 2015). However, there is also a study in which no social interaction deficit was reported following 3-NP exposure (Wiprich et al., 2020). This controversy might be attributed to the different animal model (zebrafish) and experimental conditions used in the stated study.

In addition, the duration of non-social behaviors of the animals revealed a decrease in the time spent on rearing and solitary behavior. It also showed an increase in the duration of immobility. These findings, demonstrating impaired motion activity following 3-NP injection, are consistent with previous studies (Duan et al., 2000; Thangarajan et al., 2014).

Investigations show restraint stress (Chiba et al., 2012), social defeat stress (Buwalda et al., 2001), prenatal stress (Weinstock, 2008), early-life maternal separation (Aisa et al., 2008), and low maternal care (Weaver et al., 2004; Weaver et al., 2006) can decrease GR levels in different brain

regions. This reduction is associated with psychiatric symptoms like anxiety and depression. Beyond these physical and emotional stresses, metabolic stress may also reduce the GR level in the brain and cause behavioral abnormalities.

Here, we found that 3-NP reduced the GR level which was significant in the ST and HIP regions. These findings are partly in line with those of other studies, in which the normal energetic function of mitochondria in the brain is impaired. For example, maternal hypoxia in rats causes a decrease in the GR level in the HIP of both male and female fetuses (Gonzalez-Rodriguez et al., 2014). Also, a study on adult rats demonstrated that transient ischemia in the brain reduces GR level in the HIP (48). It is reported that transient brain ischemia can also reduce the GR level in the cortex of newborn rats (Lee et al., 2007). We also found a declining trend in the amount of this receptor in the PFC; however, this was not statistically significant. It may be due to the fact that the frontal cortex consists of different subregions. Each of these sub-regions may have different biochemical capacities and reactions.

One reason for the decreased GR level may be the epigenetic and post-translational changes. It is shown that even rather milder stressors inhibit GR gene expression by inducing epigenetic changes in the GR gene promoter and raising the level of a specific microRNA that lowers the GR mRNA expression (Mifsud et al., 2017). When rats are subjected to a greater level of stress, such as progressive mitochondrial failure, this process may become even more prominent. Also, duration of treatment and chronic glucocorticoid increases can result in a compensatory downregulation of GR levels as well as a decrease in glucocorticoid binding (Burnstein et al., 1991; Dufour & McBride, 2019).

FKBP5 is another protein that is closely linked to GR signaling. Its level can mirror the efficiency of the GR signaling pathway (Menke et al., 2012; Vermeer et al., 2003). Stressors can cause

alterations in the brain by influencing the interactions between GR and FKBP5 (Rowson et al., 2024). Studies on animal models have revealed that all regions of the brain can be affected by glucocorticoid induction of FKBP5 expression (Lee et al., 2010; Merkulov et al., 2017; Scharf et al., 2011).

We found that 3-NP decreased FKBP5 levels in all the studied brain regions. Similar to our findings, two transgenic models of HD showed significantly lower FKBP5 levels in the ST (Bailus et al., 2021) and generally higher corticosterone levels (Dufour & McBride, 2016). These findings show that despite a positive correlation between glucocorticoid level and FKBP5 level, FKBP5 level drops in the brain in this context of mitochondrial failure, even though glucocorticoid level is increased.

Here, the decreased FKBP5 level can be justified through the hypothesized concept that in chronic stress, increased FKBP5 level by glucocorticoids reduces glucocorticoid effects on GR target gene expressions (like GR induction of FKBP5 biosynthesis) via restricting GR translocation to the nucleus. It has been suggested that, under chronic stress and stress-related psychological states, inappropriate downregulation of these genes contributes to glucocorticoid resistance (Merkulov et al., 2017).

Reduced GR signaling in the brain is a main pathophysiology of psychiatric disorders (Hasler, 2010; Tsigos & Chrousos, 2002). For example, post-mortem studies have shown lower GR expression levels in different brain regions of individuals with major depressive disorder (MDD) than in controls (Alt et al., 2010). Patients with MDD also have lower GR sensitivity in their peripheral tissues, which can be a proper reflection of GR sensitivity in the brain (Pariante, 2004). In rodents, many environmental factors mentioned earlier induce anxiety and depression by reducing GR signaling in the brain (Aisa et al., 2008; Weaver et al., 2004; Weaver et al., 2006;

Weinstock, 2008). Downregulation of GR in the forebrain can impair the HPA axis balance and lead to increased levels of corticosterone and depression-like behaviors (Boyle et al., 2005). 3-NP exposure also induces anxiety and depression (Khodagholi et al., 2022). The current study suggests that reduced levels of GR in the ST and HIP can be a ground for 3-NP-related social behavior impairment. Even in the PFC, where we only found a falling trend without a significant change in GR level, decreased FKBP5 level may indicate an attenuated GR signaling pathway.

Beyond anxiety and depression, social behaviors are also closely modulated by HPA axis activity. For example, restraint stress that increases HPA activity and corticosterone level (Flores et al., 1990) can impair social interaction behaviors (Zain et al., 2019). Social defeat stress also acts in the same manner (Iniguez et al., 2014). Although social behaviors have been studied in other contexts with mitochondrial failure, the mechanistic relation behind that is less known. Based on our findings, it can be hypothesized that 3-NP reduces GR levels in the brain, which may lead to HPA axis overactivity and result in impaired social behaviors.

For a more accurate assessment of the effects of complex II inhibition by 3-NP treatment, measuring key variables in the next steps of the mitochondrial respiratory chain, including kinds of cytochromes can offer a wider view of the affected processes. We observed that 3-NP reduced various cytochrome levels in different studied brain regions. Previously, the ST has been found to show a reduction in cytochrome contents following 3-NP exposure, which is in accordance with our findings (Mehrotra et al., 2015; Sandhir et al., 2014). In the current study, Cyt aa_3 and Cyt b were found to be significantly reduced in all studied regions in comparison with the control group. Cyt c and Cyt c_1 also revealed significant decreases in the ST and hippocampal regions. However, their levels did not alter in the PFC. The simultaneous reduction of Cyt b related to complex III and Cyt aa_3 of complex IV, while no change in Cyt c and Cyt c_1, probably indicates the greater

resistance of these cytochromes to 3-NP treatment in the PFC. It may also be related to the compensatory responses in PFC.

MAO is a fundamental brain enzyme located mainly on the outer membrane of the mitochondria (Youdim et al., 2006). MAO-A and MAO-B are two types of this enzyme that are involved in metabolizing monoamines (e.g., dopamine, serotonin, noradrenaline) in the brain and other tissues (Markey, 2007). According to investigations, alteration in MAO enzymatic activity may have significant impacts on controlling behavior and contribute to the pathogenesis of a wide range of mental disorders and neurodegenerative diseases, from antisocial personality disorder to Parkinson's disease (Bortolato & Shih, 2011). MAO inhibitors are therapeutic agents for improving symptoms of affective disorders, like depression (Pletscher, 1991; Yanez et al., 2012). Inhibition of this enzyme is also beneficial in the treatment of anxiety disorders like social anxiety disorder (Williams et al., 2017). However, MAO might have a different role when the neurons are under the pressure of mitochondrial failure.

In the current study, MAO-A and MAO-B activities decreased significantly in 3-NP-treated animals in ST and HIP compared to the control group. It was different from our initial expectations; since post-mortem studies of HD patients show that MAO-A and MAO-B levels are increased in their several brain regions (Richards et al., 2011). However, we found that our findings are in line with previous studies in which 3-NP administration has been shown to reduce total MAO activity in the ST (Chakraborty et al., 2014; Haider et al., 2022; Mohd Salman et al., 2022; M. Salman et al., 2022). Also, according to another study, 3-NP most likely reduces dopamine (DA) breakdown in isolated striatal nerve terminals by indirectly lowering MAO-A activity (Herrera-Mundo & Sitges, 2010).

In the lack of a clear-cut explanation for the controversies mentioned, it seems that unequal MAO-A and MAO-B responsibilities for metabolizing different monoamines in various species (Youdim et al., 2006), the different potential affinity of these enzymes, and the monoamine dynamics under mitochondrial failure in the brain (Chakraborty et al., 2014; Eradiri & Starr, 1999; Jamwal & Kumar, 2016; Johnson et al., 2000; Kraft et al., 2009; P. Kumar et al., 2010) can be the causes of these observations.

Furthermore, 3-NP can also reduce the 5-HT levels in brain regions like ST, cortex, and HIP, even when the MAO activity is decreased (P. Kumar et al., 2010; M. Salman et al., 2022). It shows that MAO activity might not be the pivotal modulator of the monoamines' dynamic in the brain. However, lower activity of these enzymes may lead to behavioral changes. For example, lower activity of the MAO-A enzyme in the human brain is related to higher aggressive behaviors, which is somehow the opposite of social behavior (Alia-Klein et al., 2008).

Also, recent studies show that MAO activity not only does not increase the cytosolic hydrogen peroxide (H2O2) but also activates the electron transport chain and ATP production by shuttling electrons to the intermembrane space of mitochondria (Graves et al., 2020). Together with our findings, it can be suggested that 3-NP-induced reduction in MAO-A and MAO-B activities prevents the cells from a potentially compensating role of MAO activity and can exacerbate mitochondrial failure. However, determining the key players of the molecular and behavioral consequences of 3-NP, still need more investigation. Study of stress or other neurotoxic effects of 3-NP, except mitochondrial dysfunction, along with behavioral changes in future research might disclose the more exact mechanism of action of this toxin.

Data availability statement: The data that support the findings of this study are available from the corresponding author if requested.

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Author Contributions: *F.N. and N.K.* carried out the experiment. *F.N., F.K.,* and *F.F.* contributed to the conceptualization and study design. *F.N., N.K., A.Z,* and *M.M.* participated in the data analysis. *F.N., A.M., A.Z., M.M., and D.M* contributed to the data interpretation and writing the manuscript. *F.K. F.F., A.M, and D.M.* contributed to editing the manuscript. *F.K. and F.F.* supervised the project. All authors read and approved the final manuscript.

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Figure Legends

Figure 1.

Comparison between social behaviors of the control and 3-NP treated group in the social interaction test. Duration of social behaviors including **A)** play behaviors, **B)** genital investigation, **C)** social-nose contact, and **D)** the total social behavior time is presented (n=8-10/group). The data is presented as mean ± SEM. ***p < 0.001.

Figure 2.

Comparison of non-social behaviors between control and 3-NP treated group in the social interaction test. Duration of non-social behaviors including **A)** rearing, **B)** self-grooming, **C)** exploring, **D)** solitary behavior, and **E)** immobility is presented (n=8-10/group). The data is presented as mean ± SEM. ***p < 0.001, ****p < 0.0001.

Figure 3.

Comparison of the GR and FKBP5 levels in the striatum, hippocampus, and prefrontal cortex between the control and 3-NP treated groups. Changes in the striatal (**A, D**), hippocampal (**B, E**), and prefrontal (**C, F**) protein ratio to β-actin (n=4/group). The data is presented as mean ± SEM. *p < 0.05, ***p < 0.001, ****p < 0.0001.

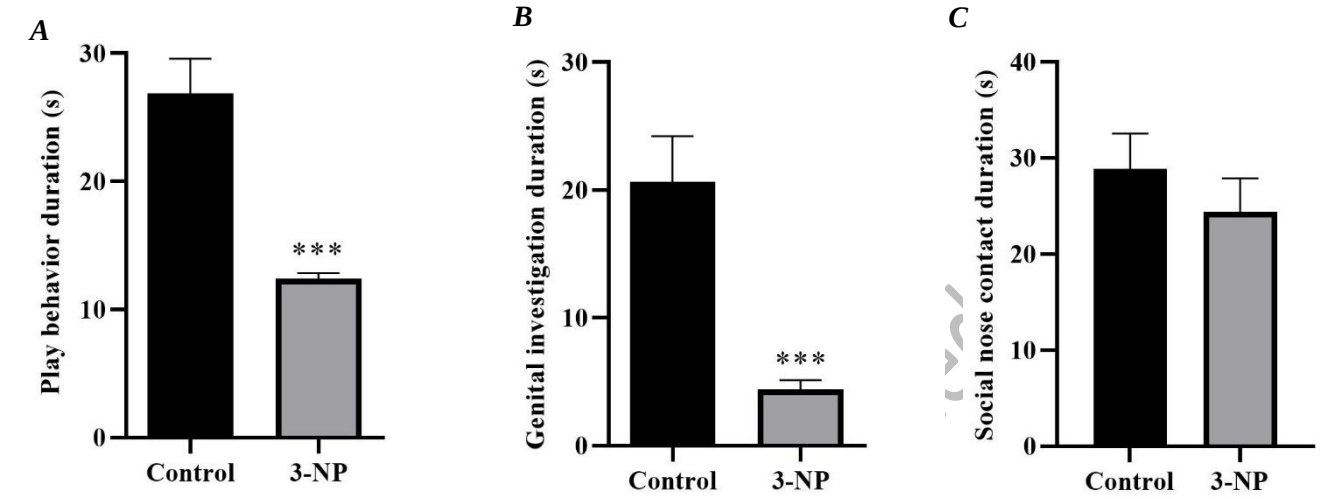
Figure 4.

Comparison of the cytochrome contents in the different studied brain regions between 3-NP treated groups and the control. The quantity of striatal (**A**), hippocampal (**B**), and prefrontal (**C**) cytochromes in the 3-NP group is presented as a percentage of the control group (n=6/group). The data is presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Figure 5.

Comparison of MAO-A and MAO-B activities respectively, in the striatum (**A, D**), hippocampus (**B, E**), and prefrontal cortex (**C, F**) between the control and 3-NP treated groups (n=6/group). The data is presented as mean ± SEM. *p < 0.05, **p < 0.01.

Figure1



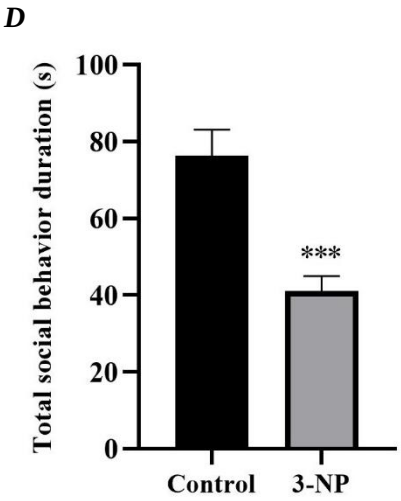


Figure 2

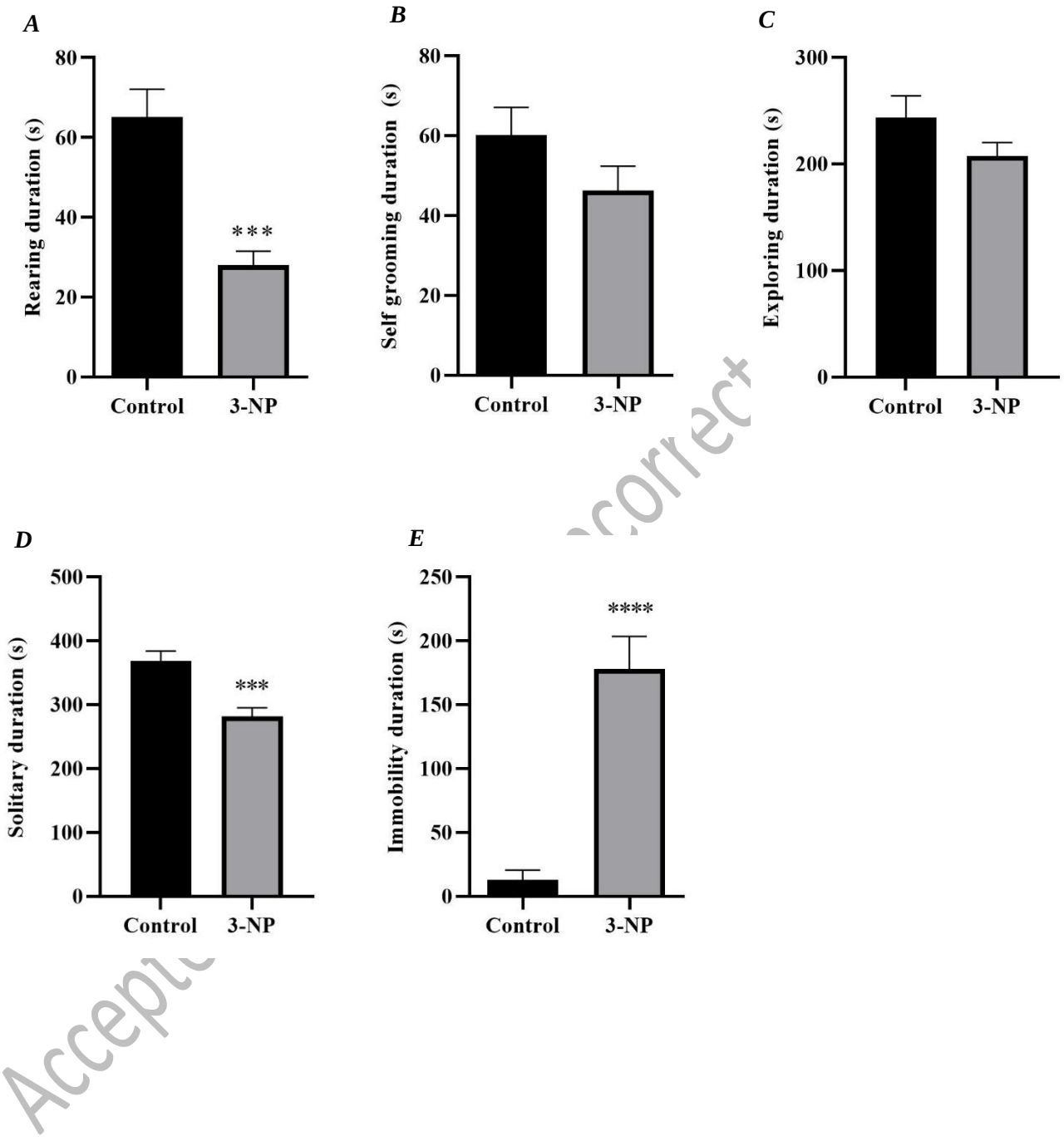


Figure 3

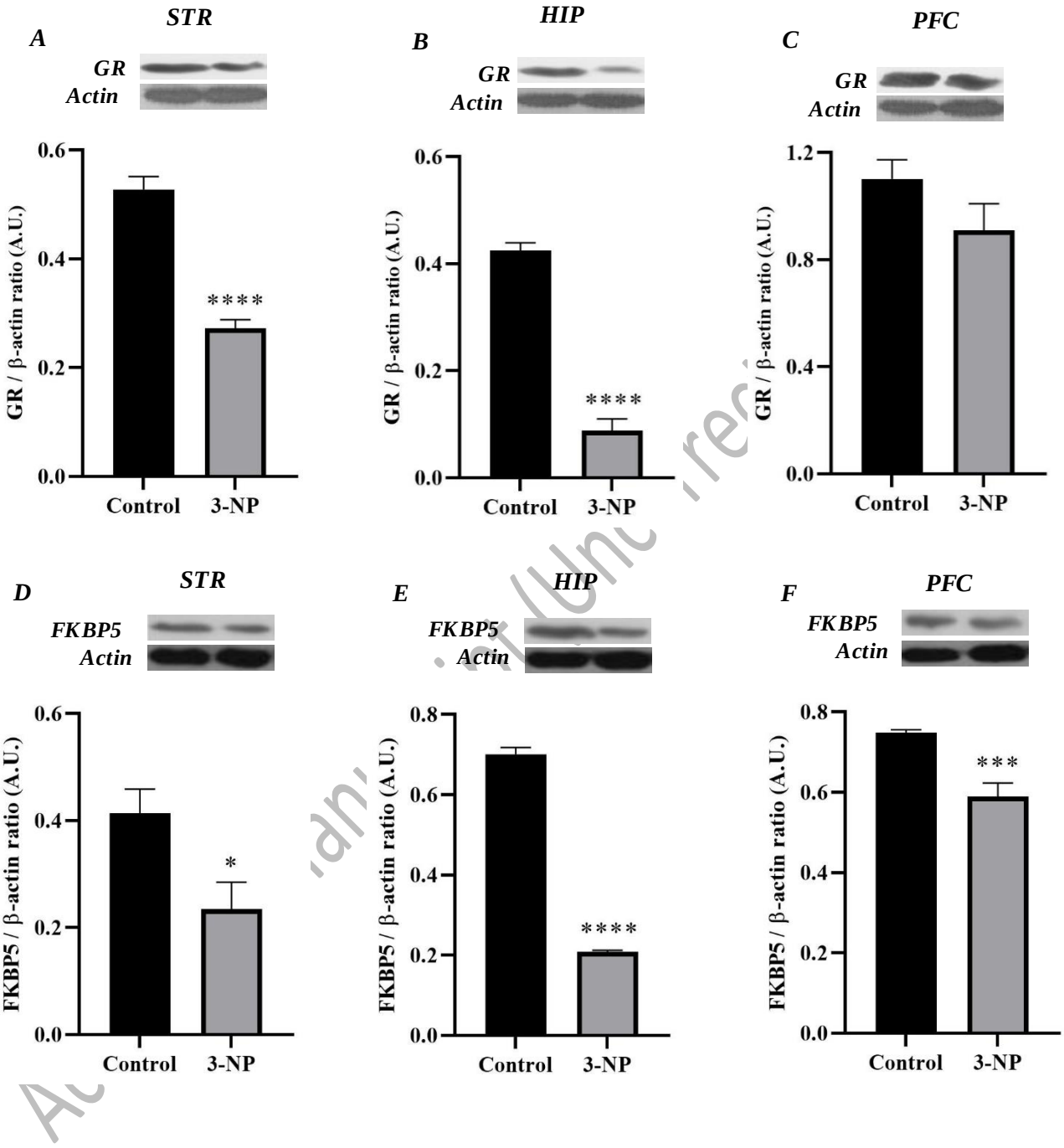

A
STR
GR
Actin
GR / β-actin ratio (A.U.)
0.6
0.4
0.2
0.0
****
Control
3-NP
B
HIP
GR
Actin
GR / β-actin ratio (A.U.)
0.6
0.4
0.2
0.0
****
Control
3-NP
C
PFC
GR
Actin
GR / β-actin ratio (A.U.)
1.2
0.8
0.4
0.0
Control
3-NP
D
STR
FKBP5
Actin
FKBP5 / β-actin ratio (A.U.)
0.6
0.4
0.2
0.0
*
Control
3-NP
E
HIP
FKBP5
Actin
FKBP5 / β-actin ratio (A.U.)
0.8
0.6
0.4
0.2
0.0
****
Control
3-NP
F
PFC
FKBP5
Actin
FKBP5 / β-actin ratio (A.U.)
0.8
0.6
0.4
0.2
0.0
***
Control
3-NP

Figure 4

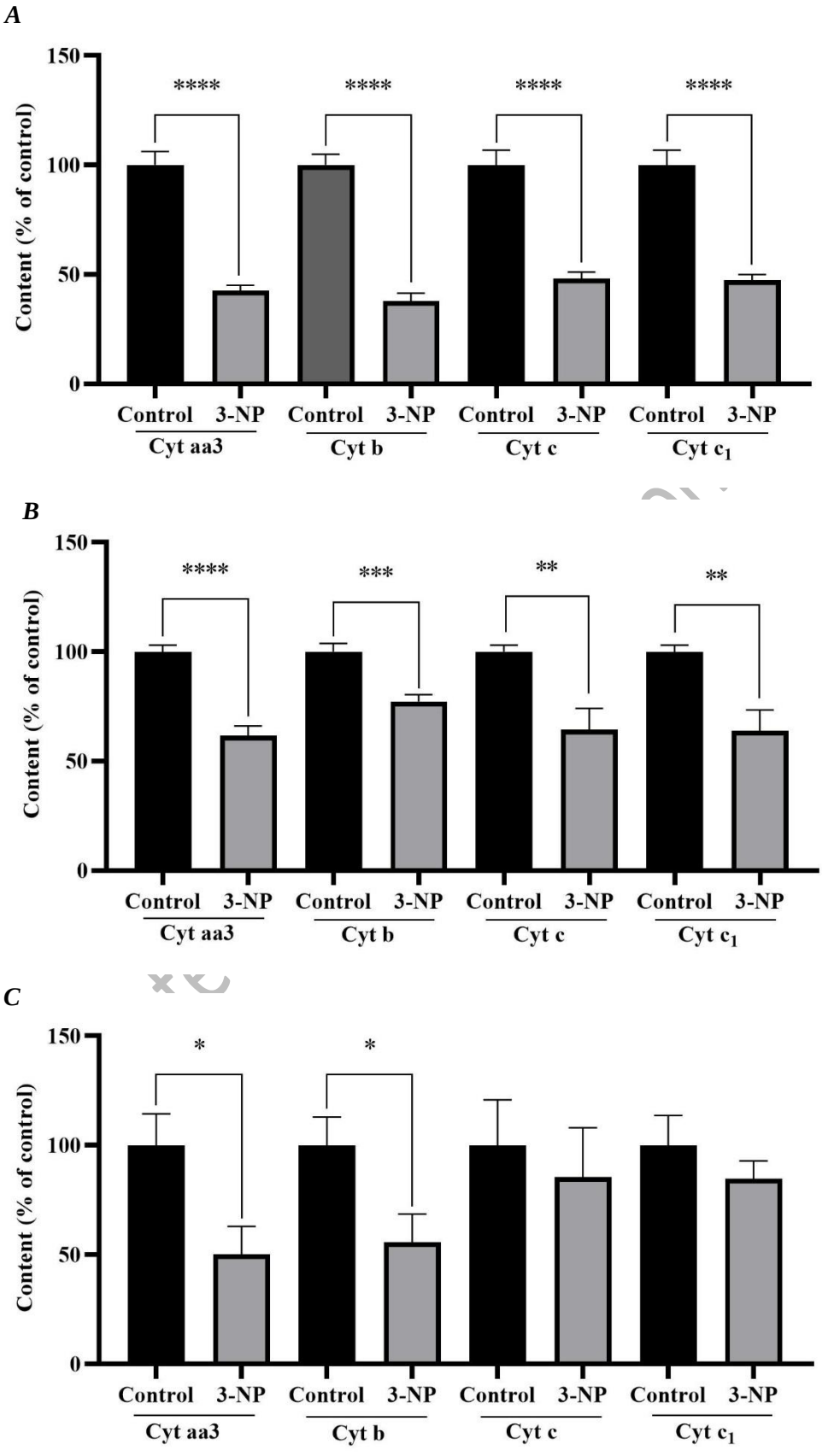

A
150
100
50
0
Content (% of control)
****
****
****
****
Control
3-NP
Control
3-NP
Control
3-NP
Control
3-NP
Cyt aa3
Cyt b
Cyt c
Cyt c1
B
150
100
50
0
Content (% of control)
****
***
**
**
Control
3-NP
Control
3-NP
Control
3-NP
Control
3-NP
Cyt aa3
Cyt b
Cyt c
Cyt c1
C
150
100
50
0
Content (% of control)
*
*
Control
3-NP
Control
3-NP
Control
3-NP
Control
3-NP
Cyt aa3
Cyt b
Cyt c
Cyt c1

Figure 5