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Full-Length Research Article

Intranasal Dexamethasone Selectively Impairs Exploratory and Learning Behaviour While Sparing Peripheral Metabolic and Oxidative Homeostasis in Adult Mice

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Summary: Dexamethasone (Dex) is a potent synthetic glucocorticoid with broad clinical applications, but systemic administration often leads to metabolic disturbances and cognitive side effects. Intranasal Dex has potential to deliver glucocorticoids directly to the brain, minimizing systemic side effects associated with traditional administration. However, its comprehensive effects on metabolic, behavioural, and neurochemical endpoints after repeated dosing remain unclear. Adult male mice were randomly assigned to four groups (5-7 mice per group): control (saline) and dexamethasone (Dex) doses of 5, 15, or 50 µg/kg administered intranasally once daily for seven days. Peripheral measures included body weight, fasting blood glucose, glucose tolerance test (GTT), fasting serum and brain insulin levels, HOMA IR, and serum corticosterone via ELISA. Behavioural assays comprised the Morris Water Maze, Elevated Plus Maze, Open Field Test, and Novel Object Recognition Test. Brain homogenates were analysed for oxidative stress markers (superoxide dismutase, glutathione), cholinergic activity (acetylcholinesterase), and trace minerals (Zn, Fe, Ca). Intranasal Dex did not affect body weight, glycaemic control, insulin sensitivity, or corticosterone levels. Dose dependent declines in locomotor exploration and spontaneous alternation were observed, alongside increased escape latencies during spatial learning without altering memory retention or novel object recognition . No significant change was observed in the levels of markers of oxidative stress and important metals like Zn, Fe, and Ca. Dexamethasone decreased central acetylcholinesterase activity, albeit insignificantly. Intranasal Dex selectively impairs exploratory behaviour and learning acquisition through central glucocorticoid receptor–mediated synaptic and cholinergic modulation with no impact on preserving peripheral glucose metabolism and central markers of oxidative stress.

Keywords*: dexamethasone, intranasal delivery, cognitive impairment, glucocorticoid receptors, synaptic plasticity,*

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INTRODUCTION

Dexamethasone (Dex) is a potent synthetic glucocorticoid widely utilized for its anti-inflammatory and immunosuppressive properties in conditions ranging from autoimmune disorders to cerebral oedema and chemotherapy-induced nausea (Reichardt et al., 2021; Vazquez et al., 2023). Despite its clinical efficacy, systemic Dex administration commonly leads to adverse metabolic outcomes, including hyperglycaemia, insulin resistance, muscle catabolism, and dyslipidaemia, as well as neuropsychiatric side effects such as mood alterations, cognitive deficits, and increased risk for neurodegenerative changes (Batista et al., 2024; Tappy et al., 1994). These off-target effects are primarily mediated through widespread activation of glucocorticoid receptors (GRs) in peripheral tissues and brain regions, prompting a search for alternative delivery strategies that can preserve therapeutic benefit while minimizing systemic burden.

Activation of GRs within hippocampal and prefrontal cortical circuits has been implicated in reductions of brain-derived neurotrophic factor (BDNF) (Chen et al., 2017; Numakawa and Kajihara, 2024) expression, dysregulation

of glutamatergic and cholinergic neurotransmission, and impairment of long-term potentiation, leading to deficits in attention, working memory, and spatial learning (Blin et al., 2023; Maurer and Williams, 2017; Paul et al., 2015; Popoli et al., 2012). Systemic Dex exposure recapitulates these effects, producing both acquisition and retrieval impairments in spatial memory tasks, elevated oxidative stress (OS) markers, and trace metal dyshomeostasis (Kim et al., 2009; Li et al., 2017; Schäfer et al., 2005; Wang et al., 2023). We have shown previously that systemic administration of Dex influences working memory and spontaneous alternation in mice (Abdulbasit et al., 2018). However, it remains unclear whether low-dose, targeted delivery of Dex can mitigate peripheral side effects while preserving central efficacy against inflammation.

Intranasal delivery offers a promising approach to bypass the blood–brain barrier, exploiting olfactory and trigeminal pathways to achieve rapid nose-to-brain transport, elevated cerebrospinal fluid concentrations, and reduced peripheral spillover (Fowler et al., 2021; Meneses et al., 2017; See, 2023). Preclinical studies confirm that intranasal steroids, including Dex, can attain therapeutic levels in brain parenchyma within minutes, with minimal systemic glucocorticoid exposure (Cárdenas et al., 2022; Meneses et al., 2017). To evaluate the safety and mechanistic impact of this route, we administered low-dose Dex intranasally to adult male mice for seven consecutive days, and comprehensively assessed peripheral metabolic parameters (body weight, fasting glucose and insulin, HOMA-IR, corticosterone), behavioural performance across exploratory, working memory, spatial learning, and recognition tasks, and neurochemical endpoints including OS markers (SOD, GSH), cholinergic activity (AChE), and trace mineral content (Zn, Fe, Ca). Thus, we aim to delineate the therapeutic window and CNS-targeted efficacy of intranasal Dex while mapping its potential to minimize systemic adverse effects.

MATERIALS AND METHOD

Animals and Grouping:

Adult male mice were used for this study. The animals were housed in standard conditions in a temperature-controlled environment with a 12-hour light/dark cycle and provided ad libitum access to food and water at the animal holding facility of the Faculty of Basic Medical Sciences, University of Ilorin, Nigeria . All protocols were approved by the University of Ilorin ethiral review committee (UIL/UERC/21/68LD001). The experimental procedures employed in this research are in line with the international guidelines for the care and use of laboratory animals.

The mice were randomly divided into four groups (5-7 mice per group). Three groups received intranasal dexamethasone (Dex) at doses of 5 µg/kg, 15 µg/kg, and 50 µg/kg body weight, respectively. The fourth group served as the control and received 2 µL of normal saline per mouse via the intranasal route. Intranasal administration was performed once daily for seven consecutive days. Prior to each administration, mice were briefly anesthetized by exposure to isoflurane vapor in a closed chamber containing isoflurane-soaked cotton wool to induce rapid and transient anesthesia, allowing for easy intranasal delivery using a micropipette.

Behavioural Assessments

Morris water maze:

After the treatment period, behavioural assessments were conducted to evaluate neurobehavioral effects. Spatial learning and memory were assessed using the Morris Water Maze (MWM). Mice were trained over 4 consecutive days (days 4, 5, 6, and 7) representing test 1, 2, & 3 and experiment to locate a hidden platform submerged in a circular pool filled with opaque water. During each trial, the mouse was released from one of three starting quadrants and allowed 60 seconds to find the platform. If successful, the mouse was allowed to rest on the platform for 10 seconds. If unsuccessful, it was guided to the platform and allowed the same resting period. On the fourth day, a probe trial was conducted (experiment) in which the platform was removed, and the time spent in the target quadrant, where the platform was previously located, was manually recorded over a 60-second period.

Elevated plus maze:

Anxiety-like behaviour was evaluated using the Elevated Plus Maze (EPM) on day 6, which consisted of two open arms and two enclosed arms arranged in a plus shape and elevated above the ground. Each mouse was placed at the junction of the arms, facing one of the open arms, and observed for five minutes. The total time spent in the open and closed arms and the number of entries into each arm were recorded manually. An entry was defined as the mouse having all four paws within an arm.

Open field test:

The Open Field Test (OFT) was conducted on day6 to assess general locomotion and exploratory behaviour. Each mouse was placed in the centre of a square open field arena, with visible markings dividing the floor into 25 equal squares. Over a five-minute observation period, the total number of squares crossed (indicative of locomotion), the time spent in the centre versus the periphery of the arena (indicative of anxiety-like behaviour), and rearing (standing on hind legs as a measure of exploratory behaviour) were manually recorded.

Novel object recognition test:

Recognition memory was assessed using the Novel Object Recognition (NOR) test on day 7. During the training phase, each mouse was placed in the open field arena with two identical objects positioned equidistant from the walls and each other. The mouse was allowed to explore the objects for five minutes, and the time spent interacting with each object was manually recorded. Following a one-hour retention interval, one of the familiar objects was replaced with a novel object. During the test phase, the mouse was reintroduced into the arena for five minutes, and the time spent exploring the novel versus the familiar object was recorded. Interaction was defined as the mouse touching or sniffing the object while oriented towards it.

Glucose tolerance test (GTT):

To investigate the peripheral metabolic effects of intranasal Dex, fasting blood glucose levels were measured on day 8 using a glucometer (AccuCheck) with blood obtained from the tail vein. Additionally, GTT was conducted after an overnight fast. Each mouse was administered glucose

intraperitoneally at a dose of 2 g/kg body weight, and blood glucose levels were measured at baseline and 15, 30-, 60-, 90-, and 120-minutes post-glucose administration .

Mice euthanasia:
At the end of the experiment, animals were euthanized using a combination of ketamine and xylazine administered intraperitoneally. Ketamine was delivered at a dose of 100 mg/kg and xylazine at 10 mg/kg to induce deep anesthesia. Adequate depth of anesthesia was confirmed by the absence of reflexive responses to paw pinch and corneal stimulation. Once animals were fully anesthetized, blood was collected via cardiac puncture. For cardiac puncture, a sterile 1 mL syringe fitted with a 23G needle was inserted into the thoracic cavity, and blood was drawn directly from the heart under aseptic conditions. Blood samples were collected into serum separator tubes and allowed to clot at room temperature for 30 minutes before centrifugation (3000 g for 15 min at 4oC) to obtain serum. Following cardiac puncture, animals were monitored to ensure cessation of heartbeat and respiration, confirming death in accordance with institutional ethical guidelines. The brains were collected afterward and homogenised in ice-cold phosphate-buffered saline and centrifuged (10,000 r.p.m for 15 min at 4oC) to collect the supernatant.

Enzyme-linked immunosorbent assay (ELISA):
Insulin was quantified in the serum and brain homogenate (supernatant) of mice using ELISA (AccuBind #5825-300). Corticosterone was also quantified in the serum (#ENZOADI900097).

Estimation of markers of Oxidative stress

Superoxide dismutase (SOD) activity assay:
SOD activity was measured in the supernatant of the brain homogenate using a commercially available colorimetric SOD assay kit (Cayman Chemical, USA #706002), following the manufacturer's instructions. The assay is based on the inhibition of xanthine oxidase–mediated reduction of nitroblue tetrazolium (NBT) to formazan. Absorbance was read at 450 nm using a microplate reader. Results were expressed as units per milligram of protein (U/mg protein).

Glutathione (GSH) assay:
Total glutathione content in the supernatant of the brain homogenates was determined using a Glutathione Assay Kit (Sigma-Aldrich, USA #CS0260) based on the reaction of GSH with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to form a yellow-colored product, 5-thio-2-nitrobenzoic acid (TNB), which was measured spectrophotometrically at 412 nm. Results were expressed as micromoles of GSH per gram of tissue (µmol/mg tissue).

Acetylcholinesterase (AChE) activity:
Acetylcholinesterase activity was quantified in the supernatant of the brain homogenates using the Ellman method, as adapted for microplate format. Briefly, the reaction mixture contained 100 µL of brain supernatant, 100 µL of 0.1 M phosphate buffer (pH 8.0), 50 µL of acetylthiocholine iodide (substrate, 1.25 mM final concentration), and 50 µL of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB, 0.5 mM) as the chromogenic reagent. The reaction was incubated at 37°C, and the increase in absorbance was monitored at 412 nm for 5 minutes using a microplate reader. AChE activity was calculated using the molar extinction coefficient of TNB (13,600 $M^{-1}cm^{-1}$) and expressed as µmol/min/mg protein.

Brain Zinc, Iron, and Calcium levels:
The concentrations of zinc (Zn), iron (Fe), and calcium (Ca) in brain homogenate supernatants were determined using a colorimetric assay kit (Sigma-Aldrich), according to the manufacturers' protocols. These assays are based on the formation of colorimetric complexes specific to each metal ion, which are quantified by measuring absorbance with a microplate reader. Zinc levels were measured based on the formation of a stable chromogen complex, with absorbance read at 560 nm. Iron concentration was determined using a ferrozine-based colorimetric reaction, with absorbance measured at 593 nm. Calcium was assessed using an o-cresolphthalein complexone (OCPC)-based method, with absorbance measured at 575–580 nm. For each element, a standard curve was prepared using the provided or certified metal standards.

Statistical Analysis
Data were expressed as mean ± standard error of the mean (SEM). Comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests for pairwise comparisons. A p-value < 0.05 was considered statistically significant. The software, GraphPad Prism v8.0 was used for statistical analyses.

RESULTS

Intranasal Dex Does Not Alter Glycaemic Control or Insulin Sensitivity:
Dexamethasone is a mimetic of endogenous corticosteroids known to induce weight gain, insulin resistance and enhance endogenous glucose production in experimental settings. We hypothesise that administering dexamethasone nasally will restrict its effect to the brain with no impact on peripheral glucose homeostasis. We administered the different doses of dexamethasone for seven days and assessed weight gain, glucose homeostasis, the fasting blood glucose levels, serum levels of corticosterone. As shown in Figure 1A, intranasal dexamethasone administration had no significant impact on the body weight for the duration of the study. Intranasal dexamethasone also did not significantly alter serum corticosterone, fasting blood glucose and serum insulin at the end of the experiment (Figure 1 B-D). To test if glucose clearance from the blood is impacted in response to glucose administration, we performed a glucose tolerance test. As shown in Figure 1 E, the rate of glucose clearance was similar across all the groups except at early time-points (15 min and 30 min) where the mice that received 5µg dexamethasone had enhanced glucose excursion. However, the experimental mice showed no difference in insulin resistance in relation to the control mice as measured by the homeostatic model for insulin resistance (HOMA-IR) (Figure 1F).

We conclude that administering dexamethasone nasally prevents the peripheral impact of dexamethasone on glucose homeostasis including insulin resistance.

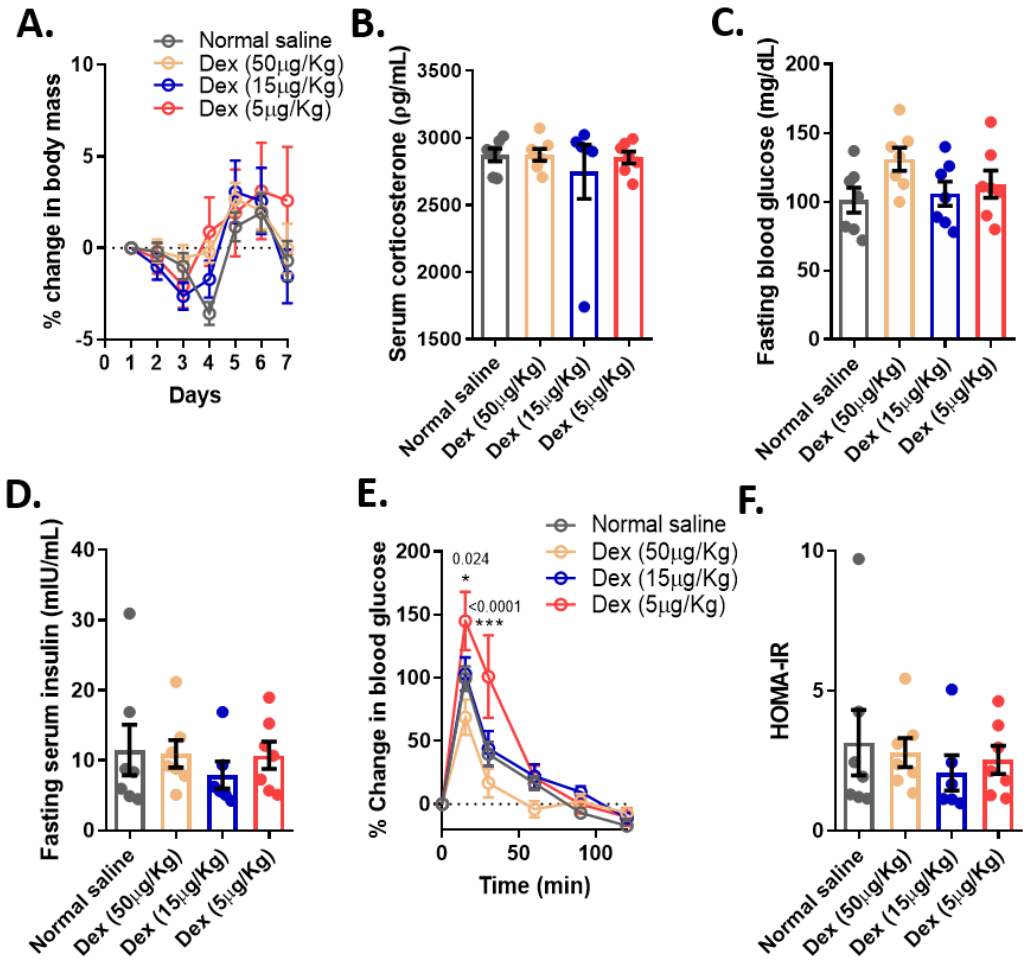

Figure 1.
Effect of intranasal Dex on body mass, corticosterone, and metabolic parameters. (A) Percentage change in body mass over a 7-day treatment period. (B) Serum corticosterone levels on day 7. (C) Fasting blood glucose concentrations. (D) Fasting serum insulin concentrations. (E) Blood glucose concentrations during GTT at 0, 15, 30, 60, 90, and 120 minutes after glucose administration. (F) HOMA-IR scores calculated from fasting glucose and insulin values. Data are presented as mean ± SEM, n = 7 animals per group. $p < 0.05$, $p < 0.001$ compared to the saline control group.

Intranasal Dex Reduces Exploratory Behaviour and Impairs Working Memory: Our results so far have shown that administering dexamethasone intranasally prevents the peripheral metabolic dyshomeostasis associated with systemic glucocorticoid administration. We thereafter tested the impact of dexamethasone on spatial working memory and exploratory behaviour in mice. The effects of Dex on spatial working memory and exploratory behavior were assessed using Y-maze spontaneous alternation task in mice. All groups treated with Dex showed a significant decrease in the total number of arm entries when compared to saline-treated controls ($p < 0.05$ to $p < 0.01$; Figure 2A), indicating a broad suppression of exploratory behavior. In addition, dexamethasone significantly decreased total alternation, the number of correct alternations, and the percentage of correct alternations in mice ($p < 0.05$ to $p < 0.01$; Figure 2 B, C, and D). These observations together show that treatment with dexamethasone significantly decreased exploration in mice and working memory

Intranasal Dex Impairs Spatial Learning but Spares Memory Retention and Recognition: After confirming that intranasal dexamethasone administration does not have an impact on peripheral glucose metabolism but impact working memory, we thereafter tested if it has an impact on spatial learning and recognition memory. The MWM test was utilized to evaluate spatial learning and memory while the NOR test was used to test recognition memory. For the MWM, 4 trials (test 1, 2, & 3, and experiment) were performed for the mice to learn the position of the hidden platform and the experiment was performed 24h after. While all the mice groups show signs of learning the platform position during these trials (Figure 3A), only the group mice that received 5 µg/kg showed no sign of memory retention and consolidation ($p < 0.001$; Figure 3A). Performance in the probe trial, which evaluates memory retention, revealed no significant differences in the time allocated to the target quadrant among the treatment groups (Figure 3B). In the NOR test, all groups treated with Dex exhibited a non-significant trend indicating reduced exploration of novel objects (Figure 3C). This pattern, while not statistically different from control ($p > 0.05$), may indicate a slight decrease in recognition memory or exploratory motivation.

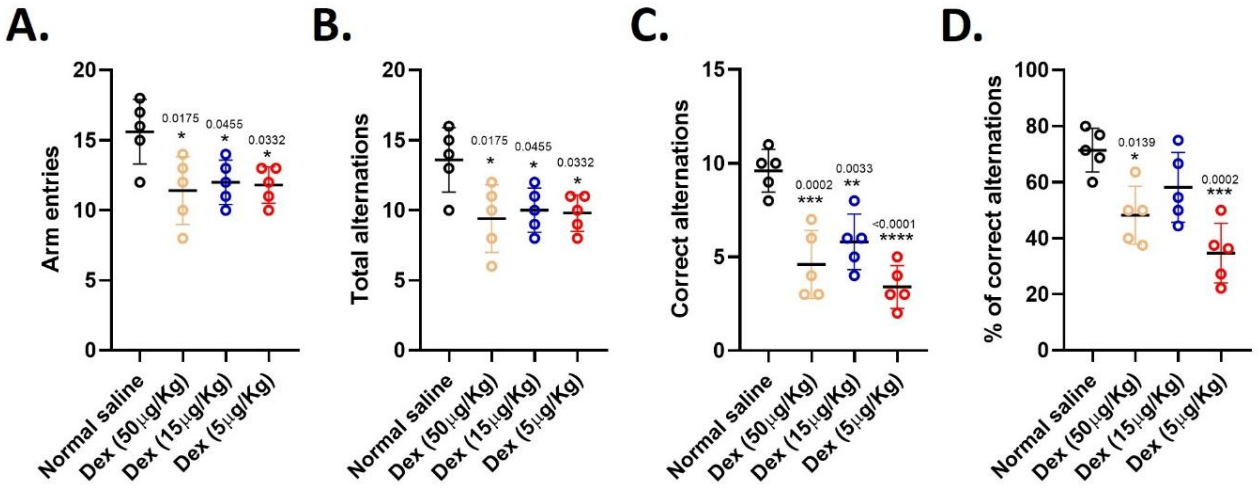

Figure 2.
Effects of intranasal Dex on working memory in the Y-maze. (A) Total arm entries. (B) Total alternations. (C) Number of correct alternations. (D) Percentage of correct alternations. Data are shown as mean ± SEM, n = 5 animals per group. $p < 0.05$, $p < 0.01$, $p < 0.001$, *$p < 0.0001$ vs. control.

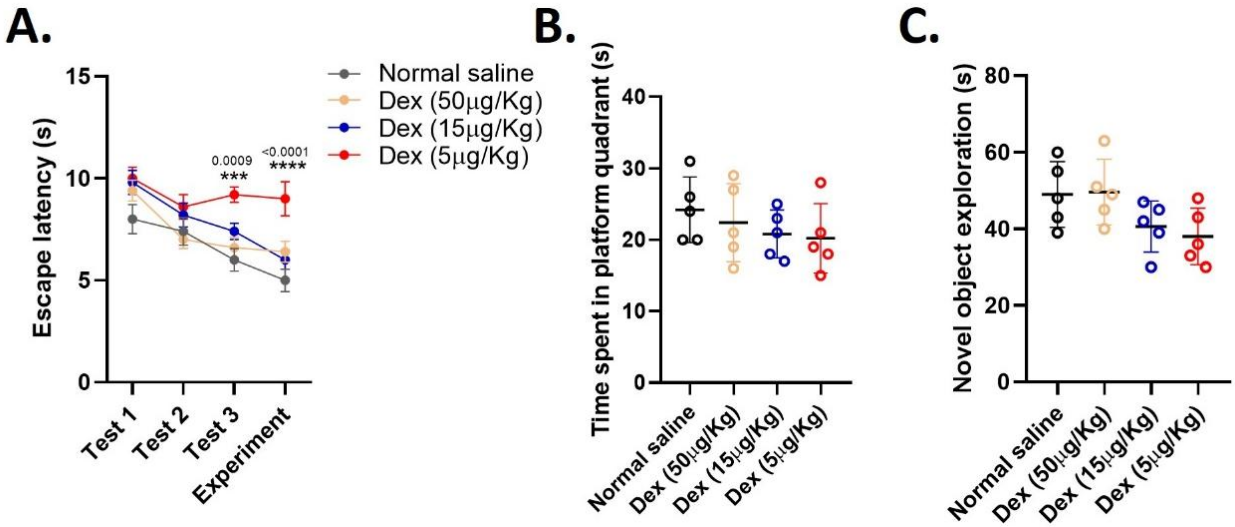

Figure 3.
Effect of intranasal Dex on learning and recognition memory. (A) Escape latency in the MWM. (B) Time in target quadrant during the probe trial. (C) Novel object exploration time in the novel object recognition test. Data are shown as mean ± SEM, n = 5 animals per group. $p < 0.01$ vs. control.

Intranasal Dex Modulates Cholinergic Activity Without Inducing Oxidative Stress or Disrupting Mineral Homeostasis: We investigated the potential neurochemical underpinnings of the observed behavioural effects by analysing oxidative stress markers, cholinergic signalling, and mineral content in brain tissue after intranasal Dex exposure. As shown in Figure 4, SOD activity and GSH levels did not show significant differences between control and treatment groups (Figures 4A and 4B; $p > 0.05$), suggesting that Dex did not induce measurable oxidative stress during the 7-day treatment period. AChE activity demonstrated a dose-dependent decline (Figure 4C); however, these reductions were not statistically significant compared to control values ($p > 0.05$). The observed downward trend may indicate early or subthreshold changes in cholinergic signalling, which could be functionally significant due to the related impairments in spatial working memory and learning.

The levels of Zn, Fe, and Ca in brain tissue remained statistically unchanged across all treatment groups (Figures 4D–F; $p > 0.05$). However, some inter-individual variability was observed in the higher dose group, especially concerning iron and calcium. The findings indicate that intranasal Dex does not substantially disrupt brain mineral homeostasis.

DISCUSSION

In this study, we evaluated the effects of a seven-day intranasal Dex regimen on both peripheral metabolic parameters and central cognitive functions in adult male mice. While measures of glucose homeostasis, insulin sensitivity, and mineral balance remained stable, we observed dose-dependent deficits in exploratory behaviour, working memory, and spatial learning, accompanied by subtle cholinergic alterations without evidence of oxidative stress or systemic HPA-axis disruption. These findings highlight the selective central impact of intranasal Dex via direct nose-to-brain pathways.

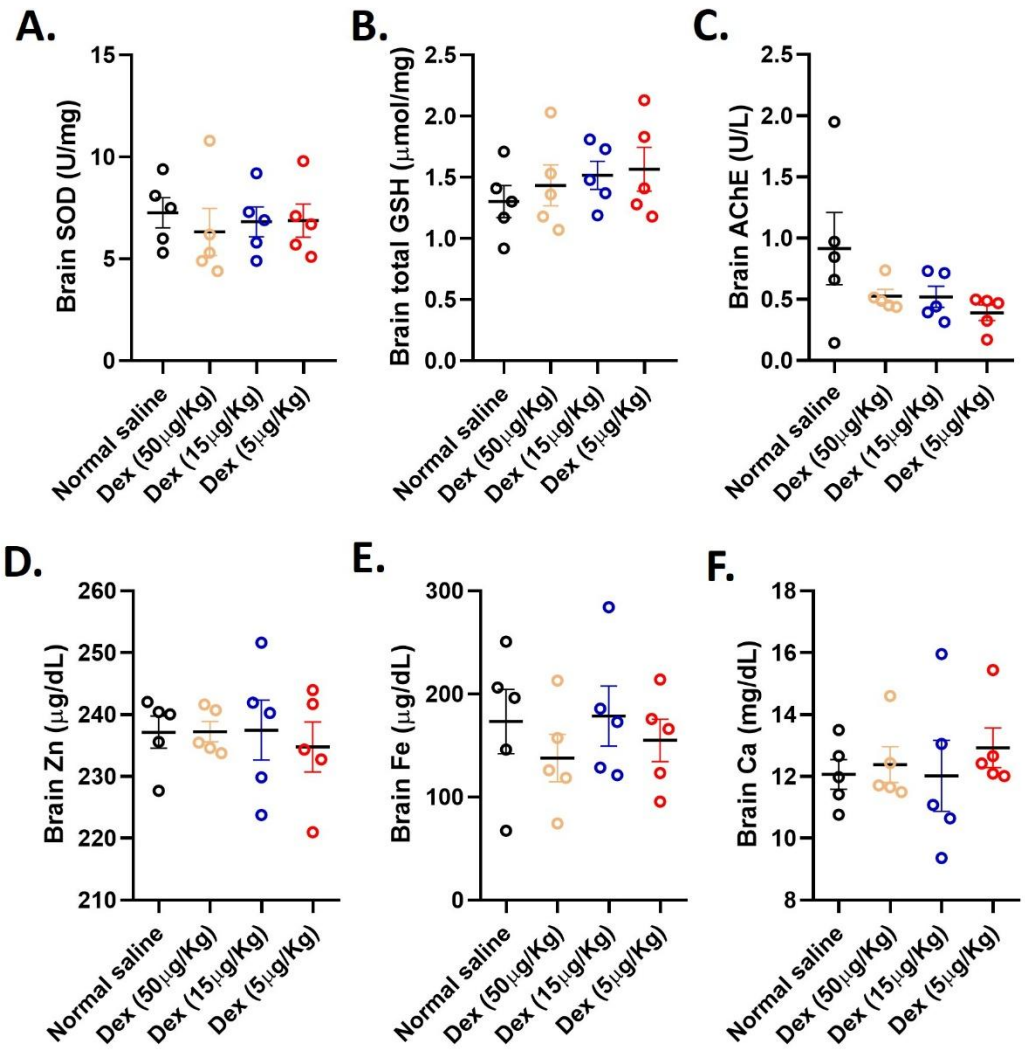

Figure 4.
Effect of intranasal Dex on oxidative stress, cholinergic activity, and mineral balance. (A) Brain SOD activity. (B) Total glutathione levels. (C) Acetylcholinesterase (AChE) activity. (D–F) Brain levels of zinc, iron, and calcium, respectively. All values are expressed as mean ± SEM, n = 5 animals per group.

Despite the potent glucocorticoid activity of Dex, intranasal administration did not alter body weight trajectories, fasting glucose or insulin levels, glucose tolerance beyond early transient peaks, HOMA IR scores, or circulating corticosterone after one week. This outcome diverges from systemic glucocorticoid protocols, which routinely provoke hyperglycaemia, insulin resistance, and HPA-axis dysregulation (Batista et al., 2024; Cho and Suh, 2024; Koorneef et al., 2022; Li and Cummins, 2022) but aligns with intranasal steroid studies demonstrating minimal peripheral effects due to efficient olfactory/trigeminal transport (Cárdenas et al., 2022; Meneses et al., 2017). Mechanistically, rapid CSF entry limits systemic exposure, thereby reducing hepatic gluconeogenesis and peripheral glucocorticoid receptor activation, which provides a metabolic-sparing advantage with potential clinical relevance for patients at risk of steroid-induced metabolic disorders (Li and Cummins, 2022).

Behaviourally, Dex-treated mice exhibited pronounced decrements in locomotion and exploratory drive, as evidenced by reduced Y maze arm entries and open-field crossings, along with dose-dependent impairments in spontaneous alternation tasks. These impairments mirror recent systemic Dex and corticosterone models reporting suppressed exploration and working memory deficits (Coburn-Litvak et al., 2003; Conrad et al., 2024; Strelow et al., 2023) yet contrast with anxiolytic-like effects observed in select intranasal budesonide studies (Hughes et al., 2003), underscoring steroid-specific and route-dependent outcomes. The concurrent reduction in exploratory behaviour and working memory performance suggests glucocorticoid receptor–mediated downregulation of brain-derived neurotrophic factor expression and impaired glutamatergic synaptic efficacy within hippocampal and prefrontal circuits (Chen et al., 2017; Numakawa and Kajihara, 2024; Popoli et al., 2012), with dendritic retraction disrupting attention and mnemonic processing.

In spatial learning assessments using the Morris Water Maze, Dex administration prolonged escape latencies during acquisition while sparing probe-trial performance, indicating interference with learning processes but retention of consolidated memory. This selective effect contrasts with chronic systemic Dex regimens that compromise both acquisition and retrieval (Coluccia et al., 2008; Jeanneteau and Coutellier, 2022; Roozendaal et al., 2004) yet resembles patterns observed in acute stress paradigms where rapid non genomic glucocorticoid signalling impairs NMDA receptor–dependent long-term potentiation (Pacheco et al.,

2017; Wang et al., 2023). The preservation of retention suggests that established synaptic changes supporting memory consolidation resist short-term Dex perturbations, informing potential timing strategies to mitigate cognitive side effects.

Neurochemically, seven days of intranasal Dex did not elevate markers of oxidative stress including SOD activity or GSH levels, nor did it disrupt zinc, iron, or calcium homeostasis, diverging from systemic exposures that often trigger redox imbalance and trace metal perturbations (Chen et al., 2020; Schäfer et al., 2005; Wang et al., 2020). However, we noted a downward trend in AChE activity, consistent with recent reports of glucocorticoid-induced cholinergic dysregulation and receptor downregulation (Kreider et al., 2005; Paul et al., 2015). Even marginal reductions in cholinergic turnover can undermine attention and working memory, suggesting that adjunctive cholinergic support may mitigate Dex-induced cognitive deficits without compromising therapeutic efficacy (Maurer and Williams, 2017).

Detailed analysis revealed low inter-individual variability in iron and calcium concentrations particularly at higher Dex doses, suggesting subtle shifts in metal compartmentalization that may influence synaptic signalling (Kim et al., 2009; Li et al., 2017). Zinc, a key modulator of NMDA receptor function and synaptic plasticity, remained stable, aligning with studies showing preserved Zn pools under acute corticosteroid exposure (Krall et al., 2022). Importantly, even minor disruptions in iron homeostasis can impact dopamine metabolism and mitochondrial function, while calcium fluctuations may alter neurotransmitter release and neuronal excitability (Ferreira et al., 2019; Wang et al., 2024). These findings underscore that intranasal Dex largely spares global mineral balance yet warrant further investigation of compartment-specific metal dynamics to fully understand its neuromodulatory effects without compromising therapeutic efficacy.

In conclusion, these results demonstrate that intranasal Dex, while sparing peripheral metabolic and oxidative systems, exerts focused effects on exploratory and cognitive functions through glucocorticoid receptor–mediated synaptic remodelling and cholinergic modulation. These mechanistic insights advocate for judicious dosing schedules and exploration of adjunctive strategies to preserve cognitive integrity in clinical applications of intranasal corticosteroids.

Several limitations of our study should be acknowledged. The seven-day treatment window may not capture longer-term effects of intranasal Dex on metabolic, behavioural, or neurochemical endpoints, and recovery or compensatory processes post-treatment remain unexplored. Manual scoring of behavioural assays introduces potential observer bias; incorporation of automated video-tracking systems would enhance objectivity and temporal resolution. Furthermore, our exclusive use of male mice limits generalizability across sexes, as sex-specific glucocorticoid responses may differ due to hormonal and receptor expression profiles. Whole-brain homogenate analyses mask region-specific alterations; future studies employing microdissection or imaging approaches are needed to delineate Dex's impact on discrete hippocampal, cortical, and subcortical circuits. Finally, while the current investigation was carried out in naïve mice, the potential impact of corticosteroids on the CNS and behaviour, especially those administered intranasally in humans should be carefully considered in patient care.

Authors' contributions:

AA conceived the idea, designed the experiment, and analysed the results. YAU, AA, and BK interpreted results, wrote and revised the first draft. SE, MOD, RMT, and FFO carried out the experiment. ALO, OA, ABN, WIA, and IA contributed to result interpretation and editing of final draft..

REFERENCES

Abdulbasit, A., Stephen Michael, F., Shukurat Onaopemipo, A., Abdulmusawwir, A. O., Aminu, I., Nnaemeka Tobechukwu, A., Wahab Imam, A., Oluwaseun Aremu, A., Folajimi, O., Bilikis Aderonke, A., Ridwan Babatunde, I., and Victor Bamidele, O. (2018). Glucocorticoid receptor activation selectively influence performance of Wistar rats in Y-maze. Pathophysiology, 25(1), 41–50. https://doi.org/10.1016/J.PATHOPHYS.2017.12.002

Batista, E. K., Lima, L. M. A. de, Gomes, D. A., Crans, D. C., Silva, W. E., Belian, M. F., and Lira, E. C. (2024). Dexamethasone-Induced Insulin Resistance Attenuation by Oral Sulfur–Oxidovanadium(IV) Complex Treatment in Mice. Pharmaceuticals, 17(6), 760. https://doi.org/10.3390/PH17060760/S1

Blin, J., Gautier, C., Aubert, P., Durand, T., Oullier, T., Aymeric, L., Naveilhan, P., Masson, D., Neunlist, M., and Bach-Ngohou, K. (2023). Psychological stress induces an increase in cholinergic enteric neuromuscular pathways mediated by glucocorticoid receptors. Frontiers in Neuroscience, 17, 1100473. https://doi.org/10.3389/FNINS.2023.1100473/BIBTEX

Cárdenas, G., Chávez-Canales, M., Espinosa, A. M., Jordán-Ríos, A., Malagon, D. A., Murillo, M. F. M., Araujo, L. V. T., Campos, R. L. B., Wong-Chew, R. M., González, L. E. R., Cresencio, K. I., Velázquez, E. G., de la Cerda, M. R., Leyva, Y., Hernández-Ruiz, J., Hernández-Medel, M. L., León-Hernández, M., Quero, K. M., Monciváis, A. S., … Sciutto, E. (2022). Intranasal dexamethasone: a new clinical trial for the control of inflammation and neuroinflammation in COVID-19 patients. Trials, 23(1), 1–11. https://doi.org/10.1186/S13063-022-06075-5/FIGURES/1

Chen, H. J. C., Yip, T., Lee, J. K., Juliani, J., Sernia, C., Hill, A. F., Lavidis, N. A., and Spiers, J. G. (2020). Restraint Stress Alters Expression of Glucocorticoid Bioavailability Mediators, Suppresses Nrf2, and Promotes Oxidative Stress in Liver Tissue. Antioxidants 2020, Vol. 9, Page 853, 9(9), 853. https://doi.org/10.3390/ANTIOX9090853

Chen, H., Lombès, M., and Le Menuet, D. (2017). Glucocorticoid receptor represses brain-derived neurotrophic factor expression in neuron-like cells. Molecular Brain, 10(1). https://doi.org/10.1186/S13041-017-0295-X,

Cho, J. H., and Suh, S. (2024). Glucocorticoid-Induced Hyperglycemia: A Neglected Problem. Endocrinology and Metabolism, 39(2), 222–238. https://doi.org/10.3803/ENM.2024.1951

Coburn-Litvak, P. S., Pothakos, K., Tata, D. A., McCloskey, D. P., and Anderson, B. J. (2003). Chronic administration of corticosterone impairs spatial reference memory before spatial working memory in rats. Neurobiology of Learning and Memory, 80(1), 11–23. https://doi.org/10.1016/S1074-7427(03)00019-4

Coluccia, D., Wolf, O. T., Kollias, S., Roozendaal, B., Forster, A., and De Quervain, D. J. F. (2008). Glucocorticoid Therapy-Induced Memory Deficits: Acute versus Chronic

Effects. The Journal of Neuroscience, 28(13), 3474. https://doi.org/10.1523/JNEUROSCI.4893-07.2008

Conrad, C. D., Peay, D. N., Acuña, A. M., Whittaker, K., and Donnay, M. E. (2024). Corticosterone disrupts spatial working memory during retention testing when highly taxed, which positively correlates with depressive-like behavior in middle-aged, ovariectomized female rats. Hormones and Behavior, 164. https://doi.org/10.1016/J.YHBEH.2024.105600

Ferreira, A., Neves, P., and Gozzelino, R. (2019). Multilevel Impacts of Iron in the Brain: The Cross Talk between Neurophysiological Mechanisms, Cognition, and Social Behavior. Pharmaceuticals 2019, Vol. 12, Page 126, 12(3), 126. https://doi.org/10.3390/PH12030126

Fowler, J., Rotenberg, B. W., and Sowerby, L. J. (2021). The subtle nuances of intranasal corticosteroids. Journal of Otolaryngology - Head and Neck Surgery, 50(1), 1–6. https://doi.org/10.1186/S40463-020-00480-Z/FIGURES/1

Hughes, K., Glass, C., Ripchinski, M., Gurevich, F., Weaver, T. E., Lehman, E., Fisher, L. H., and Craig, T. J. (2003). Efficacy of the topical nasal steroid budesonide on improving sleep and daytime somnolence in patients with perennial allergic rhinitis. Allergy: European Journal of Allergy and Clinical Immunology, 58(5), 380–385. https://doi.org/10.1034/j.1398-9995.2003.00093.x

Jeanneteau, F., and Coutellier, L. (2022). The glucocorticoid footprint on the memory engram. Current Opinion in Endocrine and Metabolic Research, 25. https://doi.org/10.1016/j.coemr.2022.100378

Kim, M. H., Lee, G. S., Jung, E. M., Choi, K. C., and Jeung, E. B. (2009). The negative effect of dexamethasone on calcium-processing gene expressions is associated with a glucocorticoid-induced calcium-absorbing disorder. Life Sciences, 85(3–4), 146–152. https://doi.org/10.1016/J.LFS.2009.05.013

Koorneef, L. L., van der Meulen, M., Kooijman, S., Sánchez-López, E., Scheerstra, J. F., Voorhoeve, M. C., Ramesh, A. N. N., Rensen, P. C. N., Giera, M., Kroon, J., and Meijer, O. C. (2022). Dexamethasone-associated metabolic effects in male mice are partially caused by depletion of endogenous corticosterone. Frontiers in Endocrinology, 13, 960279. https://doi.org/10.3389/FENDO.2022.960279/BIBTEX

Krall, R., Gale, J. R., Ross, M. M., Tzounopoulos, T., and Aizenman, E. (2022). Intracellular zinc signaling influences NMDA receptor function by enhancing the interaction of ZnT1 with GluN2A. Neuroscience Letters, 790, 136896. https://doi.org/10.1016/j.neulet.2022.136896

Kreider, M. L., Aldridge, J. E., Cousins, M. M., Oliver, C. A., Seidler, F. J., and Slotkin, T. A. (2005). Disruption of rat forebrain development by glucocorticoids: Critical perinatal periods for effects on neural cell acquisition and on cell signaling cascades mediating noradrenergic and cholinergic neurotransmitter/neurotrophic responses. Neuropsychopharmacology, 30(10), 1841–1855. https://doi.org/10.1038/SJ.NPP.1300743;KWRD=MEDICINE

Li, H., Jiang, S., Yang, C., Yang, S., He, B., Ma, W., and Zhao, R. (2017). Long-Term Dexamethasone Exposure Down-Regulates Hepatic TFR1 and Reduces Liver Iron Concentration in Rats. Nutrients 2017, Vol. 9, Page 617, 9(6), 617. https://doi.org/10.3390/NU9060617

Li, J. X., and Cummins, C. L. (2022). Fresh insights into glucocorticoid-induced diabetes mellitus and new therapeutic directions. Nature Reviews. Endocrinology, 18(9), 540. https://doi.org/10.1038/S41574-022-00683-6

Maurer, S. V., and Williams, C. L. (2017). The cholinergic system modulates memory and hippocampal plasticity via its interactions with non-neuronal cells. Frontiers in Immunology, 8(NOV), 299560. https://doi.org/10.3389/FIMMU.2017.01489/XML/NLM

Meneses, G., Gevorkian, G., Florentino, A., Bautista, M. A., Espinosa, A., Acero, G., Díaz, G., Fleury, A., Pérez Osorio, I. N., del Rey, A., Fragoso, G., Sciutto, E., and Besedovsky, H. (2017). Intranasal delivery of dexamethasone efficiently controls LPS-induced murine neuroinflammation. Clinical and Experimental Immunology, 190(3), 304. https://doi.org/10.1111/CEI.13018

Numakawa, T., and Kajihara, R. (2024). An Interaction between Brain-Derived Neurotrophic Factor and Stress-Related Glucocorticoids in the Pathophysiology of Alzheimer's Disease. International Journal of Molecular Sciences 2024, Vol. 25, Page 1596, 25(3), 1596. https://doi.org/10.3390/IJMS25031596

Pacheco, A., Aguayo, F. I., Aliaga, E., Muñoz, M., García-Rojo, G., Olave, F. A., Parra-Fiedler, N. A., García-Pérez, A., Tejos-Bravo, M., Rojas, P. S., Parra, C. S., and Fiedler, J. L. (2017). Chronic stress triggers expression of immediate early genes and differentially affects the expression of AMPA and NMDA subunits in dorsal and ventral hippocampus of rats. Frontiers in Molecular Neuroscience, 10, 274093. https://doi.org/10.3389/FNMOL.2017.00244/BIBTEX

Paul, S., Jeon, W. K., Bizon, J. L., and Han, J. S. (2015). Interaction of basal forebrain cholinergic neurons with the glucocorticoid system in stress regulation and cognitive impairment. Frontiers in Aging Neuroscience, 7(MAR). https://doi.org/10.3389/FNAGI.2015.00043

Popoli, M., Yan, Z., McEwen, B. S., and Sanacora, G. (2012). The stressed synapse: The impact of stress and glucocorticoids on glutamate transmission. Nature Reviews Neuroscience, 13(1), 22–37. https://doi.org/10.1038/NRN3138

Reichardt, S. D., Amouret, A., Muzzi, C., Vettorazzi, S., Tuckermann, J. P., Lühder, F., and Reichardt, H. M. (2021). The Role of Glucocorticoids in Inflammatory Diseases. Cells 2021, Vol. 10, Page 2921, 10(11), 2921. https://doi.org/10.3390/CELLS10112921

Roozendaal, B., Hahn, E. L., Nathan, S. V., De Quervain, D. J. F., and McGaugh, J. L. (2004). Glucocorticoid Effects on Memory Retrieval Require Concurrent Noradrenergic Activity in the Hippocampus and Basolateral Amygdala. Journal of Neuroscience, 24(37), 8161–8169. https://doi.org/10.1523/JNEUROSCI.2574-04.2004

Schäfer, S. C., Wallerath, T., Closs, E. I., Schmidt, C., Schwarz, P. M., Förstermann, U., and Lehr, H. A. (2005). Dexamethasone suppresses eNOS and CAT-1 and induces oxidative stress in mouse resistance arterioles. American Journal of Physiology - Heart and Circulatory Physiology, 288(1 57-1), 436–444. https://doi.org/10.1152/AJPHEART.00587.2004,

See, K. C. (2023). Impact of inhaled and intranasal corticosteroids on glucose metabolism and diabetes mellitus: A mini review. Http://Www.Wjgnet.Com/, 14(8), 1202–1211. https://doi.org/10.4239/WJD.V14.I8.1202

Strelow, D. N., Magalhães, L. S., Paim, M. P., Krüger, L. D., Neto, J. S. S., Brüning, C. A., and Bortolatto, C. F. (2023). Depressive-like behavior and cognitive impairment induced by acute administration of dexamethasone: Pharmacological effects of 2-phenyl-3-(phenylselanyl)benzofuran in female Swiss mice. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 125, 110772. https://doi.org/10.1016/J.PNPBP.2023.110772

Tappy, L., Randin, D., Vollenweider, P., Vollenweider, L., Paquot, N., Scherrer, U., Schneiter, P., Nicod, P., and Jéquier, E. (1994). Mechanisms of dexamethasone-induced insulin

resistance in healthy humans. The Journal of Clinical Endocrinology and Metabolism, 79(4), 1063–1069. https://doi.org/10.1210/JCEM.79.4.7962275

Vazquez, S., Gold, J., Spirollari, E., Akmal, S., and Hanft, S. J. (2023). The story of dexamethasone and how it became one of the most widely used drugs in neurosurgery. Journal of Neurosurgery, 140(4), 1191–1197. https://doi.org/10.3171/2023.9.JNS231099

Wang, J., Zhao, J., Zhao, K., Wu, S., Chen, X., and Hu, W. (2024). The Role of Calcium and Iron Homeostasis in Parkinson's Disease. Brain Sciences, 14(1), 88. https://doi.org/10.3390/BRAINSCI14010088

Wang, L., Yin, Y. L., Liu, X. Z., Shen, P., Zheng, Y. G., Lan, X. R., Lu, C. B., and Wang, J. Z. (2020). Current understanding of metal ions in the pathogenesis of Alzheimer's disease. Translational Neurodegeneration 2020 9:1, 9(1), 1–13. https://doi.org/10.1186/S40035-020-00189-Z

Wang, Y., Zhang, Y., Hu, J., Pan, C., Gao, Y., Liu, Q., Xu, W., Xue, L., and Hu, C. (2023). Glucocorticoids modulate neural activity via a rapid non-genomic effect on Kv2.2 channels in the central nervous system. Neurobiology of Stress, 28, 100593. https://doi.org/10.1016/J.YNSTR.2023.100593.