Frontiers in Scientific Research and Technology

Online ISSN: 2682-2970
Print ISSN: 2682-2962
http://fsrt.journals.ekb.eg

FSRT J 12 (2025) 93 - 104 10.21608/fsrt.2025.426266.1182

Ultrastructural Changes in the Avian Brain Wulst during Captivity: A Transmission Electron Microscopy Study

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ARTICLE INFO

Article history:
Summited 25 September 2025
Received in revised form 17 October 2025
Accepted 20 October 2025
Available online 28 October 2025

Keywords
Birds captivity,
Acute and chronic stress,
Brain wulst,
Ultrastructural and
pathological changes.

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ABSTRACT

Stress presents challenges, and without adaptation, animals may develop severe psychological and physiological disorders regulated by neural networks manifesting in change of behavior and brain morphology. This study was undertaken to examine the subcellular ultrastructural changes in the avian forebrain's Wulst under acute and chronic captivity conditions, utilized transmission electron microscopy (TEM). The study involved avian species with high (crow and myna) and low (cattle egret) cognitive abilities. Birds were maintained in captivity in standard cages for 1 day and 7 days, representing acute and chronic captivity, respectively. In both phases of captivity there were many pathological changes in the Wulst, including cytoplasmic disorganization with vacuolization and electron-dense inclusions alongside increased lysosomal and autophagic activity, mitochondrial shape changes, swelling and loss of cristae, chromatin condensation, nuclear membrane irregularities; suggesting cellular degradation and impaired energy metabolism, and indicative of oxidative stress, protein aggregation, chromatolysis, necrosis and potential apoptotic processes. However, in acute captivity, all these changes were noted but with a lesser degree of occurrence than in chronic phase, indicating preserved neuronal integrity, and intact synaptic structures. There were variations of ultrastructural changes in avian forebrain's Wulst after captivity stress on the three bird species. The Crow's Wulst was the less affected followed by Myna, while in the cattle egret there was a severe damage. These findings highlight the differential effects of captivity duration on cellular ultrastructure, with chronic stress leading to pronounced neuronal degeneration and alters the neural ultrastructure more robustly during the chronic captivity.

1. Introduction

Bird brains exhibit remarkable plasticity and adaptability, enabling survival in diverse environments. However, captivity introduces challenges that impact brain morphology and cellular structure due to stress, behavioral changes, and limited natural activities. Investigating brain changes in captivity is crucial for evaluating bird welfare and adaptability. Captivity-induced constraints can stress birds, adversely affecting welfare and compromising research outcomes (Karaer et al., 2023). The avian wulst, primarily composed of the hyperpallium and neostriatum, and in passerine songbirds, the mesopallium, has extensive reciprocal connections with the telencephalon and diencephalon (Stacho et al., 2020). This brain region is essential for vision, fine control of beak and head movements, and various cognitive functions in birds.

Additionally, avian's wulsts influence behavior and may establish neural representations or control behaviors in response to interceptive changes reflecting bodily states (Güntürkün et al., 2021; Niu et al., 2022; Ströckens et al., 2022).

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It plays a crucial role in avian behavior and adaptive responses, especially under environmental changes, linking stress with brain function. Recognizing the clinical and environmental relevance of stress can enhance our understanding of deeper brain integration (Güntürkün et al., 2024). The behavioral significance of the wulst may explain its unique cytoarchitecture and chemoarchitecture. All birds possess an avian wulst, but its absence in some classes indicates a lack of certain cognitive functions. Birds with advanced cognitive functions likely have more developed wulsts, which are sensitive to environmental stress (Gaede et al., 2023).

The hooded crow (*Corvus cornix*) is a sizable omnivorous bird belonging to the order Passeriformes, family Corvidae, and genus Corvus. It is commonly found throughout Northern, Eastern, and Southeastern Europe, as well as extensively in the Middle East (Keller et al., 2020). Known for their remarkable cognitive skills and overall intelligence, hooded crows excel at adapting to new environments (Emery & Clayton, 2004).

The common myna (*Acridotheres tristis*), a member of the Sturnidae family, is known for its sociable nature and adaptability. Its capacity to produce a range of

vocalizations to deter predators makes it an excellent subject for empirical studies (Griffin, 2008). Mynas are often kept as cage birds due to their pleasant, varied, and melodic songs, their ability to mimic human speech, their intelligence, and their attractive plumage. Consequently, many of the areas where they are now found outside their native range were initially populated by birds that escaped captivity.

The cattle egret (*Bubulcus ibis*) is a bird species within the heron family, classified under the order Pelecaniformes (Telfair, n.d. 2006). This heron, part of the Ardeidae family, is found globally and inhabits tropical, subtropical, and warm-temperate regions. The cattle egret (*Bubulcus ibis*) is prevalent in Egypt (Hering et al., 2021). In a predominantly agricultural nation like Egypt, wild birds are extensively distributed due to their ability to adapt to significant climatic variations.

Birds under adverse conditions or restraint exhibit chromosomal damage, increased oxidative stress, and reduced antioxidant defenses. Prolonged glucocorticoid elevation inhibits adrenocorticotropic hormone secretion, lowering body mass and increasing metabolic rates (Domínguez-de-Barros et al., 2024). Captivity is a chronic stressor, and understanding species' responses to captive conditions is vital for addressing welfare effects and developing interventions. Evaluating birds' stress capacity is essential for developing effective coping strategies and adaptive mechanisms (Vágási et al., 2020). Stress assessment can occur during acute phases, immediate reactions, long-term stress, recovery, and memory effects. In avian species, stress response assessment involves examination of physiological markers, behaviors, health conditions, and brain regions (Huber et al., 2021).

Electron microscopy provides structural images at a resolution sufficient to evaluate nervous system cellular structure and its potential microscale alterations. Electron microscopy has proven useful in revealing brain tissue ultrastructure and alterations at pre- and postsynaptic levels due to stress (Ibáñez et al., 2023). Electron microscopy of specific brain areas can predict functional differences in birds under chronic stress. A multilevel approach is necessary to understand the effects of acute and chronic stresses (Ksepka et al., 2020; Massen et al., 2021).

By focusing on key brain regions, including the wulst, this study sought to elucidate the effects of captivity on neuronal health and function. These findings provide valuable insights into the physiological consequences of captivity and inform strategies for enhancing animal welfare in controlled environments. This study utilized electron microscopy to examine cellular and subcellular alterations in the brains of captive avian species. Analysis of ultrastructure in captivity via electron microscopy can elucidate the impact of stress, reduce environmental complexity, and limit physical activity on neuronal and glial morphology. Furthermore, captive conditions may perturb mitochondrial dynamics, synaptic organization, and other cellular processes crucial for optimal brain function.

2. MATERIALS AND METHODS

2.1. Ethical Statement

Behavioral experiments were performed in the Department of Zoology at Suez University and approved by the Institutional Animal Care and Use Committee (No. 22224). No protected, endangered, or rare species were used, and no special authorization was required.

2.2. Bird Model

The research hypothesis depended on selection a bird model and is influenced by the cognitive abilities and versatility of crow and myna species, especially in captive settings. Furthermore, the common myna is renowned for its lively, sociable, friendly, clever, and adaptable nature, which makes it an ideal candidate for living in captivity and thriving in cages. The bird model was chosen based on its ability to flourish in both urban and rural surroundings and its cognitive abilities related to memory and learning. The current study involved six individuals (three in each phase) from each bird species, including the hooded crow (*Corvus cornix*), common myna (*Acridotheres tristis*), and cattle egret (*Bubulcus ibis*). The organisms were apprehended and confined to their native rural environments in Al-Fayoum Province before being relocated to Suez University.

This study was conducted on 18 captive birds. The birds were subjected to a standardized stress protocol according to the International Society for Applied Ethology. The birds were transported to a research laboratory at Zoology Dept., Suez University and housed in standard cages for 1 d for acutely captive birds and 7 days for chronically captive birds. They were provided with appropriate food and maintained on a 12:12-hour light/dark schedule at a room temperature of 25°C. Food deprivation was conducted prior to the trial, and *ad libitum* food and water were provided for six hours post-trial. This study was conducted between December 2021 and December 2022.

2.3. Transmission Electron Microscope Preparation (TEM)

For TEM preparation, the samples were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.0) for 2 h at room temperature, rinsed in the same buffer, and post-fixed in 1% osmium tetroxide for 2 h at room temperature. The samples were dehydrated in an ethanol series ranging from 10% to 90% for 15 min in each alcohol dilution, and finally with absolute ethanol for 30 min. The samples were infiltrated with epoxy resin and acetone through a graded series until finally in pure resin. Ultrathin sections were collected on Formvar-coated copper grids. Sections were then double stained in uranyl acetate followed by lead citrate. Stained sections were observed using a JEOL JEM 1010 transmission electron microscope at 70 kV at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University (Abdel-Aziz et al., 2017).

3. RESULTS

3.1. Ultrastructural Analysis of Cellular Morphology:

The wulst, a prominent structure in the avian forebrain, primarily composed of the hyperpallium and neostriatum, and in passerine songbirds, the mesopallium, has extensive reciprocal connections with the telencephalon and diencephalon. It is integral to sensory processing and cognitive function. Transmission electron microscopy (TEM) was used to identify pathological alterations at the subcellular level through investigating the ultrastructure of wulst hyperpallium cell types in three different bird species with high (crow and myna) and low (cattle egret) cognitive abilities subjected to acute and chronic captivity. The analysis revealed evidence of variable cellular stress, organelle dysfunction, and potential autophagic or apoptotic processes. In both phases of captivity (acute and chronic), morphological changes in cytoplasmic architecture of different cells types and disorganized cytoplasmic matrices were noted. Different changes in critical organelles, including the mitochondria, lysosomes, and notable features included densely packed regions of membrane-bound structures, and mitochondrial swelling. All of these changes were indicative of cells undergoing degeneration or response to pathological stimuli.

3.2. Ultrastructural Changes in Avian's Wulsts after Acute Captivity

In acute captivity, the cells of Crow's wulsts exhibited disorganization of the cytoplasmic organelles, with appearance of rounded electron-lucent vacuolar structures indicating potential autophagic activity. In addition, there were necrotic alterations in the cell cytoplasm, as indicated by the presence of large vacuoles, disappearance of cell organelles, and collapsed membranes. Also, swollen mitochondria with peripheral damaged membranes, small spherical mitochondria with a completely degenerated crista (cristolysis), and electron-lucent matrix were observed. In addition, large dark nuclei appeared with a detached nuclear membrane and dispersed chromatin, showing an early stage of chromatin condensation (aggregates of heterochromatin) (Fig. 1A). Moreover, many spherical-shaped mitochondria with poorly defined membranes and irregular cristae with a fine granular matrix were observed (Fig. 1B). Additionally, there were some small spherical bodies, and some altered rounded mitochondria with no cristae. Distributed mitochondria with degenerated cristae exhibited empty-spaced vacuoles or cytoplasmic inclusions with numerous vacuolations in the form of autophagosomes were also detected.

On the other hand, the wulst of acute Myna individuals showed cells with large nuclei contained large dense nucleoli and less condensation of chromatin (euchromatin and heterochromatin), and fused disrupted mitochondria as well as chromatin condensation with margination through the nuclear envelope. Moreover, enlarged nuclei with irregularly condensed chromatin and abnormal nuclear-to-cytoplasmic ratio were detected (Fig. 1C). Likewise, morphological disorders associated with injury, ischemia, or neurodegenerative disorders due to captivity that impair axonal function in the form of enlarged axons compared to normal axonal dimensions with a swelling appearance that indicates disrupted axonal transport and intracellular accumulation of organelles or debris, as shown in Figure 1D. Furthermore, layers of myelin sheath were excessive and disorganized, deviating from the compact and uniform structure of healthy myelin with disorganized axoplasm, with evidence of degeneration, such as fragmented mitochondria, lysosomes, or other organelles.

In acute captivity, the Wulst of Cattle egret individuals showed ultrastructural changes in the cytoplasm, the nuclei were enlarged and contained dense fused nucleoli. Within these nuclei, the chromatin was distributed as follows: Peripheral heterochromatin (dense, transcriptionally inactive regions) lining the nuclear envelope and euchromatin (lighter, transcriptionally active regions) occupied the central areas. In addition, in the periphery of the nuclei, there were regions occupied by dark, electron-dense heterochromatin. Also, completely apoptotic nuclei with high chromatin condensation and fragmentation were observed (Fig. 1E). Ribosomes were visible as small electron-dense granules scattered in the cytoplasm or associated with the cisternae of endoplasmic reticulum, while large electron-dense secretory granules were detected. Golgi apparatus was observed as a stack of flattened membrane-bound cisternae near the nuclei of astrocyte (Fig. 1F). Some elongated and rod-shaped mitochondria with clearly visible cristae were distributed. However, most mitochondria were rounded or spherical rather than its typical elongated, rod-shaped form, and many of them showed signs of severe structural damage, including swelling, ruptured membranes and or complete disintegration (Fig. 1E and F).

The surrounding extracellular matrix or cytoplasmic background of the hyperpallium area (wulst) of acute captive Crows appeared irregular, likely due to tissue degradation. Furthermore, prominent, large, electron-dense nuclei occupy the central portion surrounding the heavily vacuolated structures of varying sizes with cytoplasmic disintegration. The overall cellular architecture appeared to be severely compromised. Figure 2A shows two adjacent nuclei of glial cells such as astrocytes and oligodendrocyres: dark and light. The dark nucleus likely represents a cell undergoing apoptosis with an electron-dense appearance caused by chromatin condensation (pyknosis). A light nucleus represents a cell in an earlier stage of apoptosis or a potentially healthy cell. Figure 2B shows that a large, dark, electron-dense area could represent chromatin condensation, with the absence or irregularity of the nucleus, and numerous large vacuoles appearing scattered throughout the cytoplasm. Visible organelles resembling mitochondria may appear swollen with disrupted cristae. Furthermore, the surrounding extracellular matrix showed irregularities, as shown in Figure 2C. Moreover, more prominent features were observed, as shown in Figure 2D, including the presence of three dark nuclei with normal chromatin condensation, the Golgi apparatus, rough endoplasmic reticulum, and cytoplasmic vacuolations.

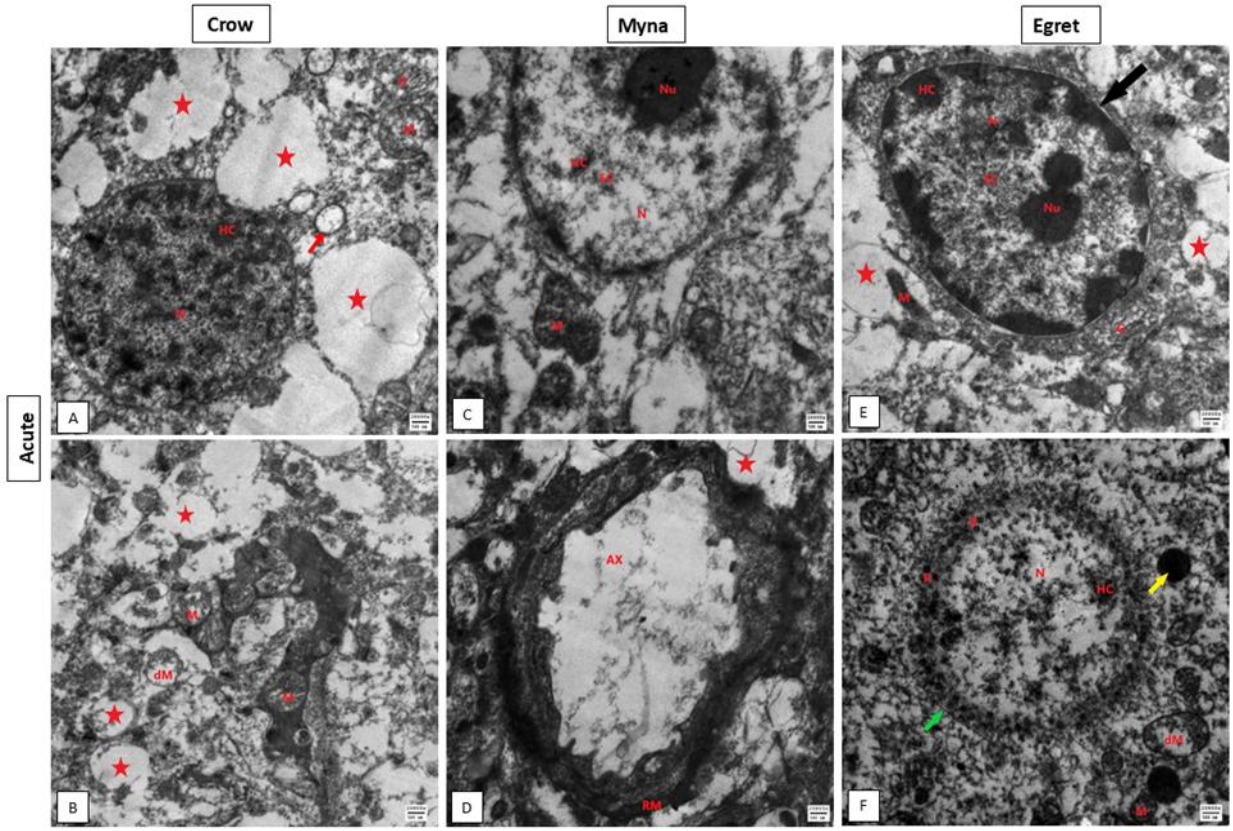

Figure 1. Transmission electron (TE) micrographs of the hyperpallium area (wulst) in the brain of acute groups of three bird species: hooded crow, common myna and cattle egret under acute captivity stress, heterochromatin (HC), nucleus (N), vacuolar structure (red star), mitochondria (M), Golgi apparatus (G), nucleolus (Nu), euchromatin (EC), mitochondrion with degenerated cristae (dM), ribosomes (R), axoplasm (AX), redundant myelin sheath (RM), small spherical mitochondria with a complete degenerated cristae (red arrow), apoptotic nucleus with chromatin condensation and fragmentation (black arrow), electron dense secretory granule (yellow arrow), and shallow dimple of nuclear envelope (green arrow, scale bar 500 nm).

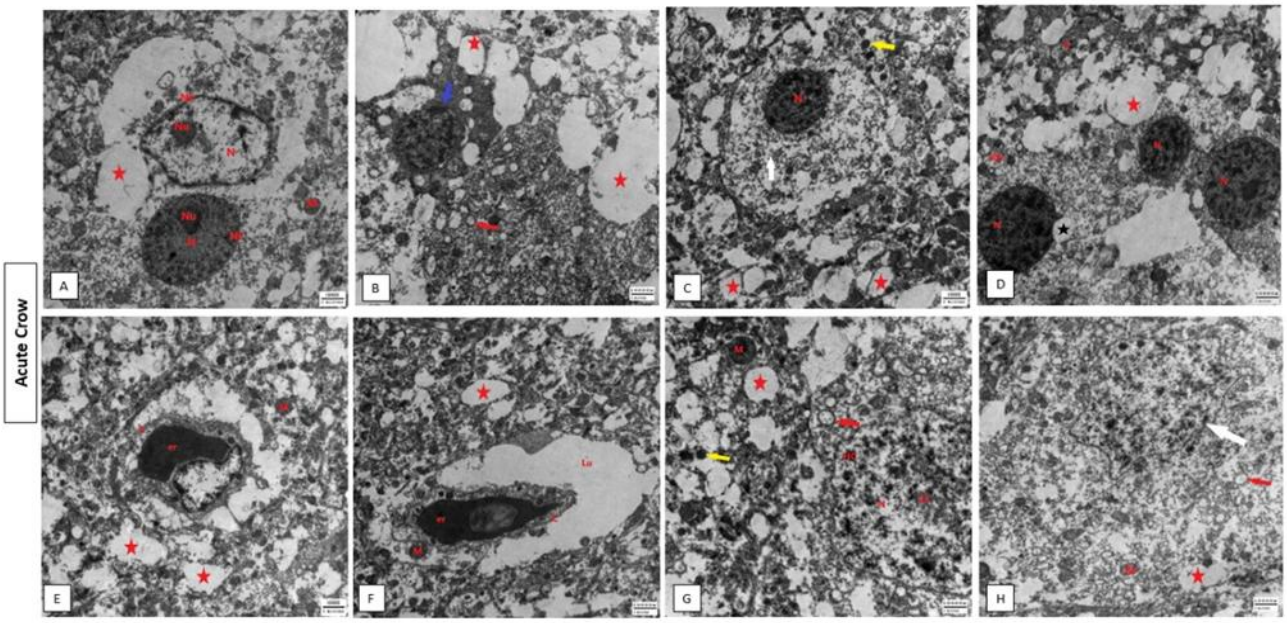

Figure 2. Transmission electron (TE) micrographs of the hyperpallium area (wulst) in the brain of hooded crow under acute captivity stress, heterochromatin (HC), nucleus (N), nuclear envelope (NE), vacuolar structure (red star), vacuolar structure contains electron-dense material (black star)mitochondria (M), Golgi apparatus (G), nucleolus (Nu), euchromatin (EC), small spherical mitochondria with a complete degenerated cristae (red arrow), electron dense secretory granule (yellow arrow), rough endoplasmic reticulum (REr), protoplasmic astrocyte (blue arrow), star- shaped neuron (white arrow), erythrocyte (er), capillary (C), and lumen (Lu, scale bar 2μm).

Additionally, there was electron microscopic appearance of normal capillaries with red blood corpuscles or erythrocytes intracapillary (Fig. 2E and F). Figure 2G shows a peripheral nucleus with less condensed chromation aggregates of euchromatin and heterochromatin with multiple distributed spherical mitochondria. Figure 2H of the ultrastructure showed irregular light star shaped nucleus indicating euchromatin dominance.

Acute Myna individuals showed disorganization in the extracellular matrix and cytoplasmic vacuoles. Nuclei were irregularly shaped with dense regions, irregular envelopes and chromatin condensation (pyknosis), with enlarged or fragmented nucleoli (Fig. 3A and B). These cells displayed swollen mitochondria with disrupted cristae, dense lysosomes and fragmented rough endoplasmic reticulum, with absent cytoskeletal elements and dark degenerated axon terminal (Fig. 3A and B). The cytoplasm contained irregular inclusions indicating protein aggregation, with large circular structures representing vacuoles, lysosomes, or electron-dense granules. Nuclei were well-defined but irregular, showing darker peripheral chromatin and lighter central euchromatin (Fig. 3C and D). Normal capillary with three red corpuscle intracapillary was observed (Fig. 3D). In other cells such as microglia, the cytoplasm appeared highly granular and packed with dense structures. The nuclei appeared oval with an irregular contour, and the nuclear membrane was discernible and irregular. Chromatin appeared with dense and dark regions in the center, possibly representing heterochromatin aggregation. Mitochondria showed signs of swelling, loss of cristae, or irregular shapes. Multiple circular, less-dense regions may represent vacuoles or swollen organelles and fragmentation or dilated cisternae of the rough ER were detected (Fig. 3F).

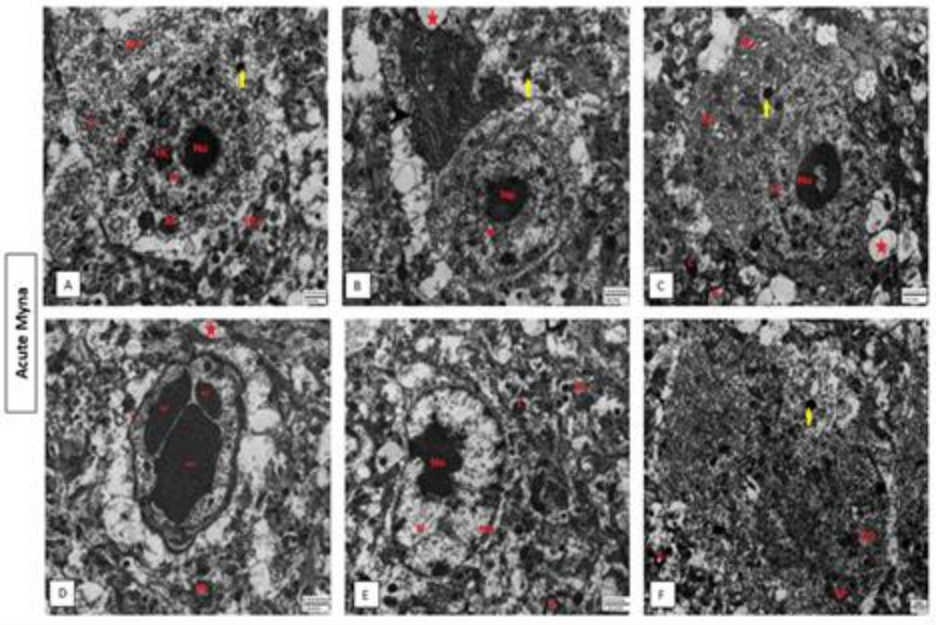

Figure 3. Transmission electron (TE) micrographs of extracellular matrix hyperpallium area (wulst) in the brain of common myna under acute captivity stress, heterochromatin (HC), nucleus (N), nuclear envelope (NE), vacuolar structure (red star), mitochondria (M), Golgi apparatus (G), nucleolus (Nu), small spherical mitochondria with a complete degenerated cristae (red arrow), erythrocyte (er), capillary (C), electron dense secretory granule (yellow arrow), rough endoplasmic reticulum (REr), lysosome (L), axon (A), and degenerated axon terminal (black arrowhead, scale bar 2µm).

In Cattle egret's wulst during acute captive conditions, the cytoplasm of cells of extracellular matrix revealed distributed areas filled with vesicular or vacuolated structures, disrupted organization, and irregular fragmented areas with cellular degeneration, necrosis and apoptosis. Besides, mitochondria, ribosomes, lysosomes, or other organelles were not distinctly visible revealed dense inclusion of the nucleus in the form of a central dark area, lacking chromatin organization and a defined envelope with heterochromatin as well as dark degenerated axon terminl (Fig. 4A and B). Figure 4C reveals Nuclear Features with a lighter central region, likely representing a nucleus with a damaged nucleolus with irregularly distributed chromatin and no distinction between euchromatin and heterochromatin. The nuclear membrane was not clearly defined, suggesting possible nuclear damage or alterations. In other cells, the cytoplasm was dense with scattered vesicles and vacuoles without clear organelle boundaries, such as mitochondria or endoplasmic reticulum, which were identifiable, and the overall organization appears disrupted. Figure 4C shows the lipofuscin granules. Figure 4D shows the blood capillary enclosed endothelial cell with its organelles and a dark patched erythrocyte in the center, with some axons surrounded by dark segments of myelin sheath forming synapses with each other. Figures 4E shows that the

nucleus of pyramidal neuron has a well-defined boundary and an overall circular oval shape without chromatin condensation, and distributed darker regions within the nucleus indicata slightly clumped heterochromatin in the stage of chromatolysis. In other cells, the cytoplasm contained dense, dark structures scattered throughout and the overall cytoplasmic organization appeared altered, with electron-dense lysosomes and vacuolated areas and lack of recognizable organelles. Additionally, prominent damaged or fragmented mitochondria appeared as dense structures.

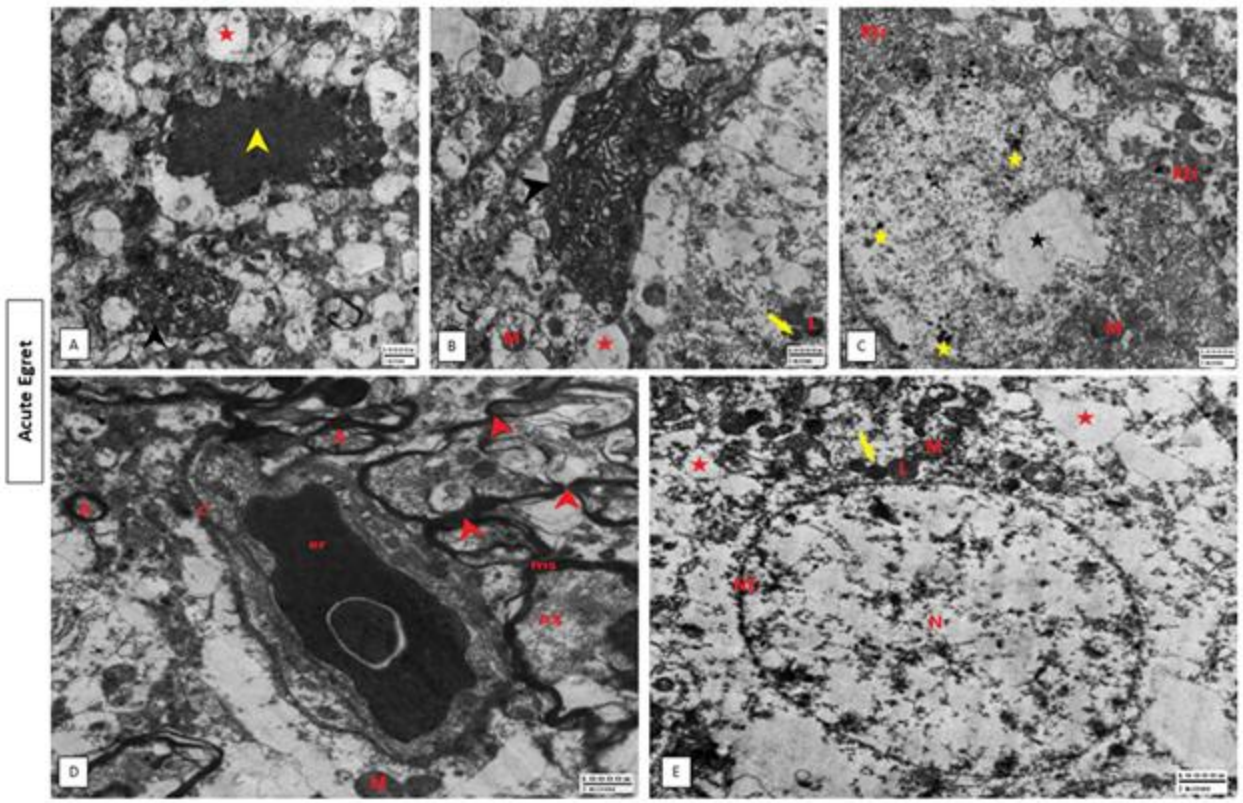

Figure 4. Transmission electron (TE) micrographs of extracellular matrix hyperpallium area (wulst) in the brain of cattle egret under acute captivity stress, nucleus (N), nuclear envelope (NE), vacuolar structure (red star), mitochondria (M), small spherical mitochondria with a complete degenerated cristae (red arrow), erythrocyte (er), capillary (C), electron dense secretory granule (yellow arrow), rough endoplasmic reticulum (REr), lysosome (L), axoplasm (AX), vacuolar structure contains electron-dense material (black star), apoptotic neuron (yellow arrowhead), degenerated axon terminal (black arrowhead), axon (A) myelin sheath (ms), synapse (red arrowhead), and lipofuscin granule (yellow star, scale bar 2μm).

3.3. Ultrastructural changes in avian' wulsts after chronic captivity:

In chronic captivity of Crows, the cytoplasm of hyperpallium area (wulst) cells contained small, spherical mitochondria, indicative of altered mitochondrial morphology and nuclei with prominent radiating nucleoli and clumps of heterochromatin (Fig. 5A). Perineuronal vacuolation or edema appeared as clear halo or space surrounding neuron, which is a characteristic feature often associated with inflammation or neuronal damage and dysfunction may be caused by activation of glial cells, and small spherical mitochondria were also visible in the cytoplasm (Fig. 5B). Figure 5C displayed cytoplasmic alterations, including the presence of few vacuolations and swollen spherical mitochondria. Spherical mitochondria with degenerated, disrupted, or completely lost cristae were also evident, indicating advanced mitochondrial damage (Fig. 5D).

Highlighting a dendrite within the cellular microenvironment, showed structural integrity or degeneration depending on the condition, necrotic alterations took place in the cell cytoplasm, indicated by the presence of large vacuoles, disappearance of cell organelles, and collapsed membranes (Fig. 5E). Furthermore, the cytoplasm contained vacuoles and mitochondria at various stages of morphological change, including dividing mitochondria, elongated forms, and complete fusion. Features of astrocyte such as an inverted pear-shaped nucleus surrounded by a shallow dimple of the nuclear envelope as sign of necrosis, and nuclei exhibited a high degree of chromatin condensation, with clumps of heterochromatin, mitochondria with longitudinally oriented cristae were visible along with abundant ribosomes were also investigated (Fig. 5F).

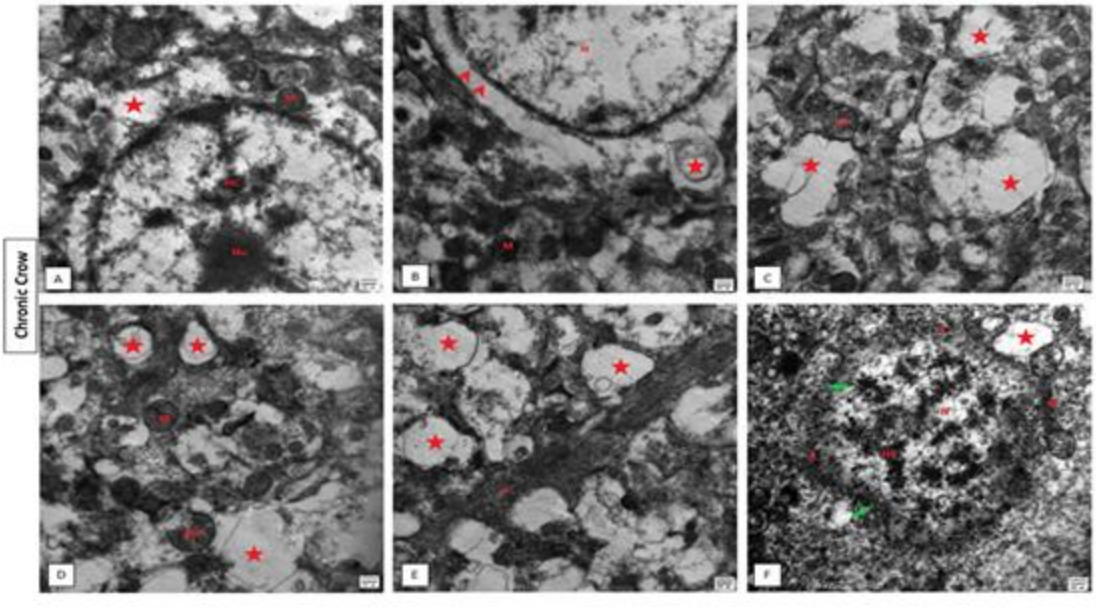

Figure 5. Transmission electron (TE) micrographs of the hyperpallium area (wulst) in the brain of hooded crow under chronic captivity stress, nucleus (N), heterochromatin (HC),vacuolar structure (red star),mitochondria (M), ribosome (R), nucleolus (Nu), mitochondrion with degenerated cristae (dM), dendrite (d), nuclear halo around neuron (double red arrowheads), dendrite (d), and shallow dimple of nuclear envelope (green arrow, scale bar 500 nm).

Ultrastructural observations in chronic Myna individuals revealed that the cytoplasm exhibited disrupted mitochondria, characterized by morphological irregularities consistent with mitochondrial dysfunction. Apoptotic features, including granules and neuron-like structures, were visible suggesting ingoing cellular degeneration or programmed cell death (Fig. 6A and B). The nuclei exhibited large, dark morphology with prominent nucleoli containing distinct, rounded chromatin clumps such as apoptotic bodies, indicative of a high degree of chromatin condensation. Heterochromatin was densely aggregated along the nuclear envelope, further emphasizing nuclear structural changes. Some membrane and cytosolic disruptions were also evident in these cells (Fig. 6 A, B, and D).

Ultrastructural observations of wulsts of chronic Cattle egrets revealed variable structures. There were alterations with extensive cytoplasmic vacuolations, indicative of severe cellular stress. Nuclear features in the form of a distributed necrotic nuclei included chromatin degradation and fragmentation in irreversible cell injury, leading to cellular swelling, membrane rupture, and the release of intracellular contents, which trigger an inflammatory response, The mitochondria were enlarged and spherical, deviating from their typical elongated morphology (Fig. 6C). Additionally, the nuclei contained a dark, electron-dense area with an irregular, amorphous material. (Fig. 6D). Moreover, some cell showed apoptotic nuclei, a high degree of vacuolations in the cytoplasm, and swollen spherical mitochondria. The nuclei contained large, rounded, and distinctly bounded chromatin clumps or showed large, dark, electron-dense areas with irregular, amorphous material (Fig. 6D).

In other areas of the (wulst) hyperpallium of chronic hooded Crow, the cytoplasm exhibited numerous circular structures such as vesicles, vacuoles, and lipid droplets. The nuclei were visible with some chromatin evenly dispersed, the nuclear membrane was intact, and mitochondria appeared swelled, losing cristae, or showed changes in the density of its matrix. Also, electron-dense regions (heterochromatin) within the nuclei were evident (Fig.7A). Furthermore, dense bodies may represent lysosomes or lipofuscin granules, which are involved in cellular degradation, were noted (Fig. 7B). In some areas of the hyperpallium, myelinated fibers were visible as dark rings with lighter central areas, representing axonal structures surrounded by myelin sheaths with a large dark staining dendrite (Fig. 7C). Enlarged axons or vacuolation around myelin sheaths, dense areas in the neuropil with irregular fibrillary material might indicate gliosis; Electron-lucent spaces around blood vessels may suggest edema (Fig. 7C). Moreover, dense black deposits may also correspond to abnormal protein aggregates, such as amyloid-like fibrils or other misfolded protein (Fig. 7B and C).

In other (wulst) hyperpallium areas of chronic common Myna, the cellular cytoplasm contained numerous organelles and ribosomes. These structures of astrocytes appear densely packed, which are typical of metabolically active cells. It also revealed dark central regions, likely representing the nucleoli. The cell organelles dispersed in the cytoplasm are distinctly visible including rough endoplasmic reticulum, Golgi apparatus, lysosome and mitochondria. The nuclei appeared intact, without obvious signs of structural disruption surrounded by nuclear envelopes (Fig. 8A).

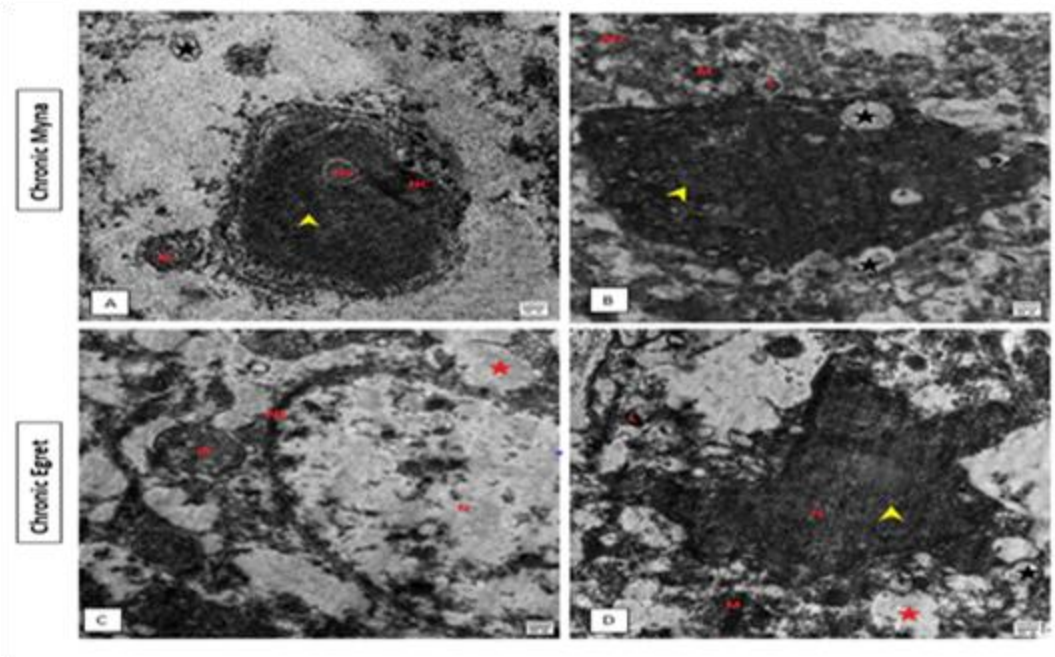

Figure 6. Transmission electron (TE) micrographs of the hyperpallium area (wulst) in the brains of common myna and cattle egrets under chronic captivity stress, nucleus (N), nucleolus (Nu), nuclear envelope (NE), heterochromatin (HC), rough endoplasmic reticulum (REr), lysosome (L), mitochondria (M), apoptotic neuron (yellow arrowhead) and some vacuolar structures are empty (red star) and some contain electron-dense material (black star), scale bar 500 nm).

Neurophagia or phagocytosis is shown where neurons are engulfed and eliminated by other cells, particularly glial cells like microglia as sign of pathology, such as during brain injury or neurodegenerative diseases (Fig. 8B). Also, in Figure 8B reveals a dark, irregularly shaped structure in the cell centre, possibly indicating abnormal aggregation or disrupted organelles. Its morphology suggests potential pathological alterations, such as loss of membrane integrity or accumulation of dense material. The surrounding cytoplasmic region appears disorganized compared to that in healthy cells. There may be signs of vacuolization or disrupted organelle arrangement, which could be indicative of cellular stress or degeneration.

Swollen mitochondria, disrupted membranes, or vacuolization could indicate cellular stress or apoptosis (Fig. 8A and C). Aggregates, vacuoles, or disrupted endoplasmic reticulum could also suggest a cellular pathology.

In other areas of the (wulst) hyperpallium of chronic cattle Egret, the cytoplasm central regions contained dense accumulations of membrane-bound structures, possibly mitochondria or vesicles (Fig. 9A). The surrounding cytoplasmic matrix appeared less organized and contained scattered organelles, indicating cellular degeneration or autophagic activity. There was a concentrated region of membrane-bound compartments the surrounding regions appeared granular with disrupted cellular integrity (Fig. 9B). Figure 9A and B reveals that dark structures are likely regions of high electron density beside the necrotic nucleus. Based on their appearance, these structures could represent swelling, loss of cristae, or condensation, which can make them appear irregularly shaped or darker than usual. Dark aggregates might form as clusters of improperly folded proteins or damaged organelles or may be dense lipid droplets or glycogen granules. Chromatolysis was showed which characterized by the dissolution of Nissl bodies (rough endoplasmic reticulum and ribosomes) in the cell body of a neuron (Fig. 9C). The cytoplasmic vacuolation were excessively distributed in the hyperpalluim of cattle egret group under chronic captivity giving the spongy appearance.

4. DISCUSSION

Animals exposed to stress exhibit behavioral problems, experience difficulties in learning and memory, and show alterations in neurological pathways and neural structures. Additionally, environmental conditions such as captivity and extended periods of confinement for experimental purposes can trigger stress responses in animals when they are removed from their natural environments (Näslund, 2021; Norman and Brando, 2024). Captivity-induced stress arises from both the absence of natural habitats and chronic stress conditions, with long-term care factors being established sources of stress (Coleman, 2021; Warwick et al., 2023). Chronic stress in captive animals can profoundly modify brain structure and function, resulting in maladaptive behaviors and neuropathological defects, impairing spatial reference and working memory (Conrad, 2010).

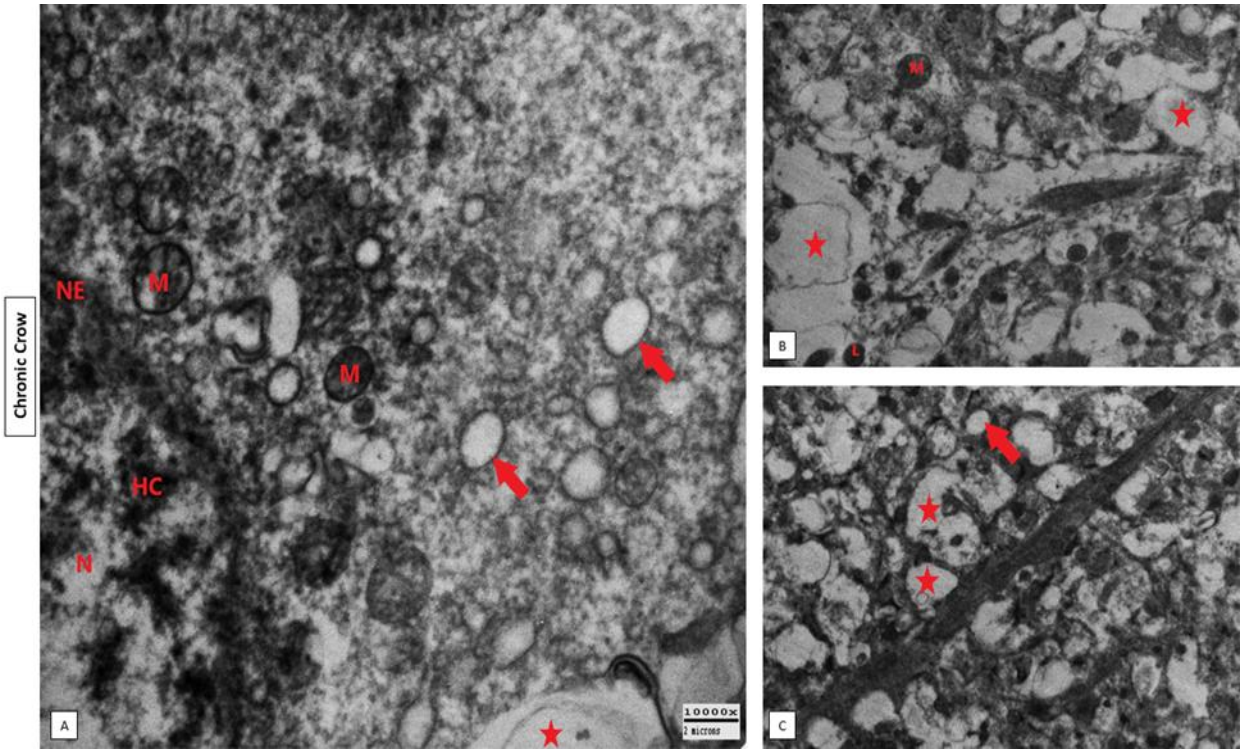

Figure 7. Transmission electron (TE) micrographs of the hyperpallium area (wulst) in the brain of hooded crow under chronic captivity stress, heterochromatin (HC), nucleus (N), nuclear envelope (NE), lysosome (L), small spherical mitochondria with a complete degenerated crista (red arrow), vacuolar structure (red star) and mitochondria (M),scale bar 2µm).

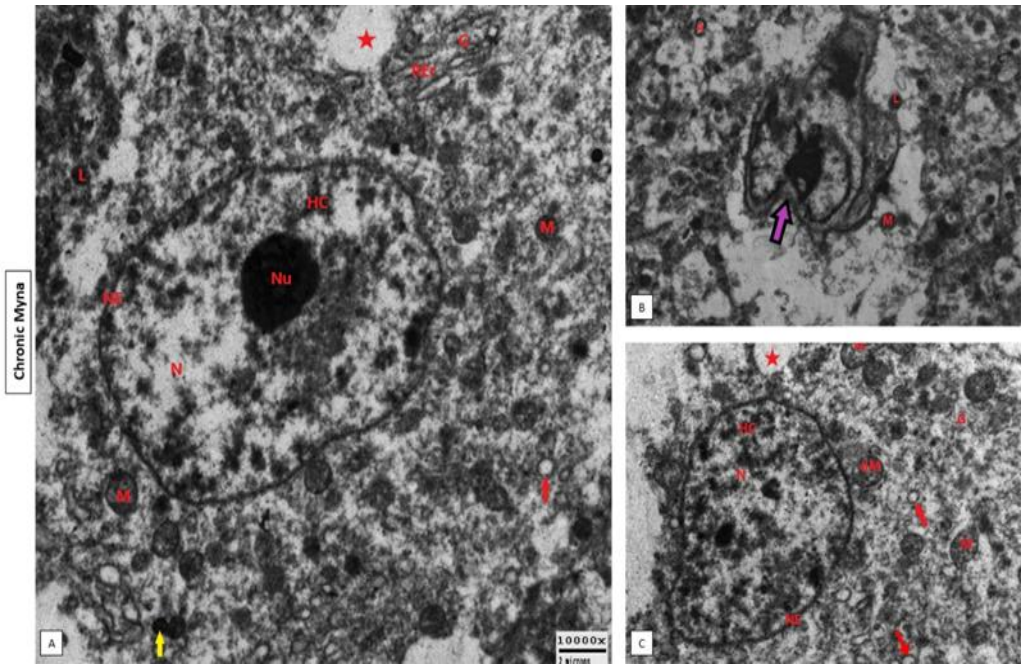

Figure 8. Transmission electron (TE) micrographs of the hyperpallium area (wulst) in the brain of common myna under chronic captivity stress, heterochromatin (HC), nucleus (N), nuclear envelope (NE), vacuolar structure (red star), mitochondria (M), Golgi apparatus (G), nucleolus (Nu), small spherical mitochondria with a complete degenerated cristae (red arrow), Golgi apparatus (G), axon (A), electron dense secretory granule (yellow arrow), rough endoplasmic reticulum (REr), lysosome (L), neurophagia or phagocyosis (purple arrow, scale bar 2µm).

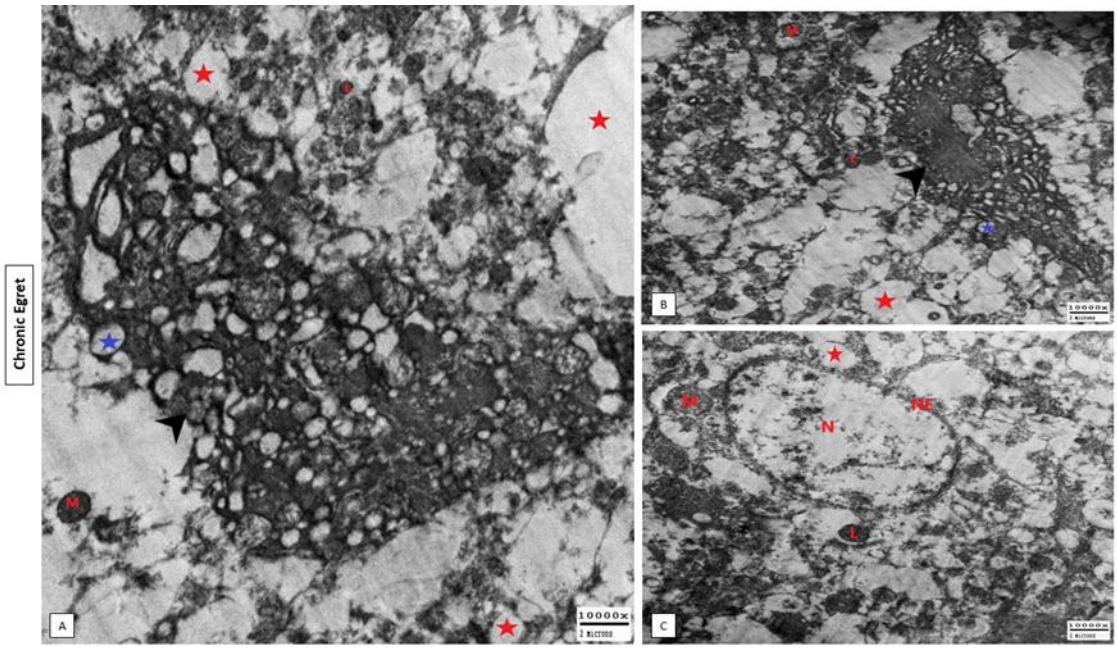

Figure 9. Transmission electron (TE) micrographs of the hyperpallium area (wulst) in the brain of cattle egret under chronic captivity stress, nucleus (N), nuclear envelope (NE), vacuolar structure (red star), mitochondria (M), small spherical mitochondria with a complete degenerated cristae (red arrow), lysosome (L), degenerated axon terminal (black arrowhead) and some agranular synaptic vesicles (blue star, scale bar 2µm).

The present study revealed distributed pathological ultrastructure in the hyperpallium wulst resulting from acute and chronic stress; however, no specific pathological manifestations were observed among these structures depending on the type of captivity. Crows in both phases exhibited less atrophy of the hyperpallium neuropil in captivity. This observation indicated a reduced level of synaptic activity and information processing. Other Passeriformes in comparable captive conditions demonstrate astrogliosis in Wulst. Furthermore, distributed lipofuscin deposition and mitochondrial degeneration (Gangloff and Greenberg, 2023) were notable in both the captive phases. Early deterioration of synaptic mitochondria is critical in synapse failure, but research on neuronal ultrastructure after captivity-induced stress is limited. The study observed scattered double-banded microfilaments and minor ultrastructural changes, maintaining neuronal integrity, healthy mitochondria, and clear nuclear membranes. Synaptic structures remained unaffected, suggesting brief acute captivity stress had minimal impact on cellular organization. However, long-term captivity causes significant ultrastructural changes, indicating neuronal stress and deterioration. Mitochondria showed enlargement and loss of cristae, suggesting compromised energy metabolism. Increased autophagy and cellular breakdown were indicated by abundant lysosomes and autophagic vacuoles. Signs of apoptosis included chromatin condensation and irregular nuclear membranes. The cytoplasm showed disorganized organelles, vacuolization, and many electron-dense inclusions, indicating oxidative damage and protein aggregation. These observations highlight gradual ultrastructural decline in Wulst during extended captivity, likely due to chronic stress and reduced environmental stimuli. Such changes may compromise neuronal function and affect sensory processing and cognitive behavior. These findings emphasize the need for enriched environments to counteract negative effects of captivity on avian brain health as suggested by Iuchi et al. (2021) and Leyane et al. (2022).

Ultrastructural changes in the cytoplasm, such as disorganization and vacuolization, are hallmarks of cellular stress, injury, and metabolic dysfunction. These changes often result from acute and chronic insults to the cell, including oxidative stress, energy depletion, or toxic stimuli exposure (Eskelinen, 2005). A disorganized cytoplasmic architecture, with loss of organelle and cytoskeletal structural integrity, reflects cellular dysfunction. This is often accompanied by ribosome redistribution or clumping, reduced protein synthesis, and impaired intracellular trafficking, suggesting disruption of normal cellular homeostasis, often preceding irreversible damage and cell death. Moreover, vacuolization indicates autophagic activity or lysosomal dysfunction. It may serve as a protective mechanism during early stress but becomes pathological when excessive. These features collectively indicate impaired energy metabolism, protein misfolding, oxidative stress, and an inability of the cell to maintain ionic and osmotic balance. Prolonged stress or failure to recover from these ultrastructural changes often culminates in apoptotic or necrotic cell death (Lemasters and Nieminen, 1997). Moreover, spherical mitochondria with poorly defined outer membranes and irregular cristae were observed in cattle egret and myna species in both captivity phases. The mitochondrial matrix appears fine and granular, reflecting potential metabolic stress or early degenerative processes. Such alterations in mitochondria have been associated with disrupted energy production

and increased susceptibility to oxidative damage under various pathological conditions (Bernardi et al., 1999). Green et al. (2011) explained that these changes are indicative of early mitochondrial dysfunction, which is often observed under conditions of cellular stress or environmental challenges during critical developmental stages. The irregular cristae and compromised membrane integrity suggest a loss of mitochondrial efficiency, which could impair ATP production and calcium homeostasis, both of which are critical for normal neuronal development and function (Kroemer et al., 2007; Wallace, 2008).

Examining structural changes in euchromatin and heterochromatin offers insights into cellular stress responses. Peripheral heterochromatin aggregation along the nuclear envelope may increase, reflecting nonessential transcription shutdown to conserve energy. Heterochromatin forms dense aggregates near the nuclear periphery or as irregular clumps within the nucleus. Chromatin condensation is a hallmark of apoptosis and senescence (Miyoshi et al., 2004). This reflects prolonged captivity stress, leading to reduced gene expression, altered nuclear organization, and programmed cell death. Chromatin aggregation can signal activation of stress-related transcriptional programs like DNA repair, autophagy, or inflammation. These chromatin patterns, visible under transmission electron microscopy, help distinguish acute stress-induced reversible changes from chronic stress-induced irreversible damage (Almeida et al., 2011).

Distributed dense fibrillary aggregates among all species could result from reactive gliosis, where astrocytes and microglia proliferate and alter their structure. The presence of such material indicates astrocytic hypertrophy and increased glial fibrillary acidic protein (GFAP) expression, characteristic of gliosis. This is commonly seen in response to neuroinflammation or injury (Pekny and Pekna, 2004). Sofroniew (2009) stated that the distributed electron-dense material or abnormal fibrillary structures under TEM may be marked for gliosis due to the glial cell proliferation and an increase in extracellular matrix proteins. And gliosis can either support recovery or exacerbate damage depending on the animal state and ability, as it involves neuroprotective as well as neurotoxic mechanisms

5. CONCLUSION

The present study concluded that acute captivity showed few alterations in the cellular ultrastructure of the Wulst in the examined bird species, with preservation of neuronal integrity, synaptic structures were largely intact, suggesting that short-term environmental stress had limited effects on cellular organization. In contrast, chronic captivity induced severe ultrastructural changes indicative of neuronal stress and degeneration. The cellular cytoplasm of Wulst exhibited disorganized organelles, vacuolization, and increased electron-dense inclusions, indicative of oxidative damage and protein aggregation. Furthermore, the mitochondria displayed swelling and loss of cristae, indicative of impaired energy metabolism. Lysosomes and autophagy were abundant, suggesting increased autophagic activity and cellular degradation. Chromatin condensation and nuclear membrane irregularities were observed, potentially reflecting apoptosis. These changes may impair neuronal function, ultimately affecting sensory processing and cognitive behaviours. There were variations of ultrastructural changes in avian forebrain's Wulst after captivity stress on the three bird species. The Crow’s Wulst was the less affected followed by Myna, while in the cattle egret there was a severe damage.

6. RESEARCH LIMITATION

The study on captivity-induced stress in avian species has several limitations. First, its findings should be generalized to other species of birds and multiple brain areas besides the hyperpallium wulst's (HW) which homology to the mammalian prefrontal cortex. Confounding factors like diet or social interactions in captivity are unaddressed. Behavioral and cognitive deficits are not directly linked to ultrastructural changes, weakening causal inferences. Reversibility of changes upon environmental enrichment is unexplored. Technical limitations of TEM, such as quantifying dynamic processes, restrict analysis. Species-specific stress adaptations are not considered, and control groups (e.g., natural or enriched environments) are undefined, complicating isolation of stress effects. The study’s narrow focus on the HW overlooks other brain regions, and ethical concerns regarding prolonged captivity are unaddressed. These limitations highlight the need for broader, more comprehensive research.

Conflict of interest: We have no Conflict of Interest.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Funding details: This work was not supported by funding agencies**.**

References

Abdel-Aziz, M. M., Yosri, M., & Amin, B. H. (2017). Control of imipenem resistant-Klebsiella pneumoniae pulmonary infection by oral treatment using a combination of myco synthesized Ag-nanoparticles and imipenem. *Journal of Radiation Research and Applied Sciences*, *10*(4), 353–360. http://inis.iaea.org/search/search.aspx?orig_q=RN:51094397

Almeida, D. M., Piazza, J. R., Stawski, R. S., & Klein, L. C. (2011). The speedometer of life: Stress, health and aging. In *Handbook of the psychology of aging* (pp. 191–206). Elsevier.

Bernardi, P., Scorrano, L., Colonna, R., Petronilli, V., & Di Lisa, F. (1999). Mitochondria and cell death. Mechanistic aspects and methodological issues. *European Journal of Biochemistry*, *264*(3), 687–701. https://doi.org/10.1046/j.1432-1327.1999.00725.x

Coleman, M. (2021). Mistreatment of Wild Animals in Captivity. *Ballard Brief*, *2021*(2), 11.

Conrad, C. D. (2010). A critical review of chronic stress effects on spatial learning and memory. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *34*(5), 742–755. https://doi.org/10.1016/j.pnpbp.2009.11.003

Domínguez-de-Barros, A., Sifaoui, I., Dorta-Guerra, R., Lorenzo-

Morales, J., Castro-Fuentes, R., & Córdoba-Lanús, E. (2024). DNA damage (8-OHdG) and telomere length in captive Psittacidae birds with different longevity. *Frontiers in Veterinary Science*, *11*, 1430861.

Emery, N. J., & Clayton, N. S. (2004). The mentality of crows: convergent evolution of intelligence in corvids and apes. *Science*, *306*(5703), 1903–1907.

Eskelinen, E.-L. (2005). Maturation of autophagic vacuoles in mammalian cells. *Autophagy*, *1*(1), 1–10.

Gaede, A. H., Wu, P.-H., & Leitch, D. B. (2023). Discrete somatotopic representation and neural response properties in hummingbird and zebra finch forebrain nuclei. *BioRxiv*, 2010–2023.

Gangloff, E. J., & Greenberg, N. (2023). *Biology of Stress BT - Health and Welfare of Captive Reptiles* (C. Warwick, P. C. Arena, & G. M. Burghardt (eds.); pp. 93–142). Springer International Publishing. https://doi.org/10.1007/978-3-030-86012-7_4

Green, D. R., Galluzzi, L., & Kroemer, G. (2011). Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science (New York, N.Y.)*, *333*(6046), 1109–1112. https://doi.org/10.1126/science.1201940

Griffin, A. S. (2008). Social learning in Indian mynahs, Acridotheres tristis: the role of distress calls. *Animal Behaviour*, *75*(1), 79–89.

Güntürkün, O., Pusch, R., & Rose, J. (2024). Why birds are smart. *Trends in Cognitive Sciences*, *28*(3), 197–209.

Güntürkün, O., von Eugen, K., Packheiser, J., & Pusch, R. (2021). Avian pallial circuits and cognition: a comparison to mammals. *Current Opinion in Neurobiology*, *71*, 29–36.

Hering, J., Fischer, S., Geiter, O., Wobker, J., & Siegel, S. (2021). Breeding colonies of gull-billed tern Gelochelidon nilotica and little tern Sternula albifrons on Lake Nasser, Egypt. *Sandgrouse*, *43*, 194–215.

Huber, N., Mahr, K., Tóth, Z., Szarka, E. Z., Çınar, Y. U., Salmón, P., & Lendvai, Á. Z. (2021). The stressed bird in the hand: Influence of sampling design on the physiological stress response in a free-living songbird. *Physiology & Behavior*, *238*, 113488.

Ibáñez, F. G., Halvorson, T., Sharma, K., McKee, C. G., Carrier, M., Picard, K., Vernoux, N., Bisht, K., Deslauriers, J., & Lalowski, M. (2023). Ketogenic diet changes microglial morphology and the hippocampal lipidomic profile differently in stress susceptible versus resistant male mice upon repeated social defeat. *Brain, Behavior, and Immunity*, *114*, 383–406.

Iuchi, K., Takai, T., & Hisatomi, H. (2021). Cell death via lipid peroxidation and protein aggregation diseases. *Biology*, *10*(5), 399.

Karaer, M. C., Čebulj-Kadunc, N., & Snoj, T. (2023). Stress in wildlife: comparison of the stress response among domestic, captive, and free-ranging animals. *Frontiers in Veterinary Science*, *10*, 1167016.

Keller, V., Herrando, S., Voříšek, P., Franch, M., Kipson, M., Milanesi, P., Martí, D., Anton, M., Klvanová, A., & Kalyakin, M. V. (2020). *European breeding bird atlas 2: distribution, abundance and change*.

Kroemer, G., Galluzzi, L., & Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. *Physiological Reviews*, *87*(1), 99–163.

Ksepka, D. T., Balanoff, A. M., Smith, N. A., Bever, G. S., Bhullar, B.-A. S., Bourdon, E., Braun, E. L., Burleigh, J. G., Clarke, J. A., & Colbert, M. W. (2020). Tempo and pattern of avian brain size evolution. *Current Biology*, *30*(11), 2026–2036.

Lemasters, J. J., & Nieminen, A.-L. (1997). Mitochondrial oxygen radical formation during reductive and oxidative stress to intact hepatocytes. *Bioscience Reports*, *17*(3), 281–291.

Leyane, T. S., Jere, S. W., & Houreld, N. N. (2022). Oxidative stress in ageing and chronic degenerative pathologies: molecular mechanisms involved in counteracting oxidative stress and chronic inflammation. *International Journal of Molecular Sciences*, *23*(13), 7273.

Massen, J. J. M., Hartlieb, M., Martin, J. S., Leitgeb, E. B., Hockl, J., Kocourek, M., Olkowicz, S., Zhang, Y., Osadnik, C., & Verkleij, J. W. (2021). Brain size and neuron numbers drive differences in yawn duration across mammals and birds. *Communications Biology*, *4*(1), 503.

Miyoshi, N., Uchida, K., Osawa, T., & Nakamura, Y. (2004). A link between benzyl isothiocyanate-induced cell cycle arrest and apoptosis: involvement of mitogen-activated protein kinases in the Bcl-2 phosphorylation. *Cancer Research*, *64*(6), 2134–2142.

Näslund, J. (2021). Reared to become wild-like: addressing behavioral and cognitive deficits in cultured aquatic animals destined for stocking into natural environments—a critical review. *Bulletin of Marine Science*, *97*(4), 489–538.

Niu, X., Jiang, Z., Peng, Y., Huang, S., Wang, Z., & Shi, L. (2022). Visual cognition of birds and its underlying neural mechanism: a review. *Avian Research*, *13*, 100023.

Norman, M., & Brando, S. (2024). The Concept of Agency, Animal Wellbeing, and the Practical Realities of Ex Situ Breeding Programs in Zoos and Aquariums. *Journal of Zoological and Botanical Gardens*, *5*(4), 563–578.

Pekny, M., & Pekna, M. (2004). Astrocyte intermediate filaments in CNS pathologies and regeneration. *The Journal of Pathology*, *204*(4), 428–437. https://doi.org/https://doi.org/10.1002/path.1645

Sofroniew, M. V. (2009). Molecular dissection of reactive astrogliosis and glial scar formation. *Trends in Neurosciences*, *32*(12), 638–647.

Stacho, M., Herold, C., Rook, N., Wagner, H., Axer, M., Amunts, K., & Güntürkün, O. (2020). A cortex-like canonical circuit in the avian forebrain. *Science*, *369*(6511), eabc5534.

Ströckens, F., Neves, K., Kirchem, S., Schwab, C., Herculano-Houzel, S., & Güntürkün, O. (2022). High associative neuron numbers could drive cognitive performance in corvid species. *Journal of Comparative Neurology*, *530*(10), 1588–1605.

Telfair, I. I. (n.d.). RC & RAYMOND, C. 2006. Cattle egret (Bubulcus ibis). *The Birds of North America Online (A. Poole, Ed.). Cornell Lab. Ornithol., Ithaca. Http://Bna. Birds. Cornell. Edu/Bna/Species/113 (Last Access on 29 January 2008)*.

Vágási, C. I., Tóth, Z., Pénzes, J., Pap, P. L., Ouyang, J. Q., & Lendvai, Á. Z. (2020). The relationship between hormones, glucose, and oxidative damage is condition and stress dependent in a free-living passerine bird. *Physiological and Biochemical Zoology*, *93*(6), 466–476.

Wallace, D. C. (2008). *A Mitochondrial Paradigm for Metabolic and Degenerative Diseases, Aging, and Cancer*. Wiley Online Library.

Warwick, C., Arena, P. C., & Burghardt, G. M. (2023). *Health and welfare of captive reptiles*. Springer.