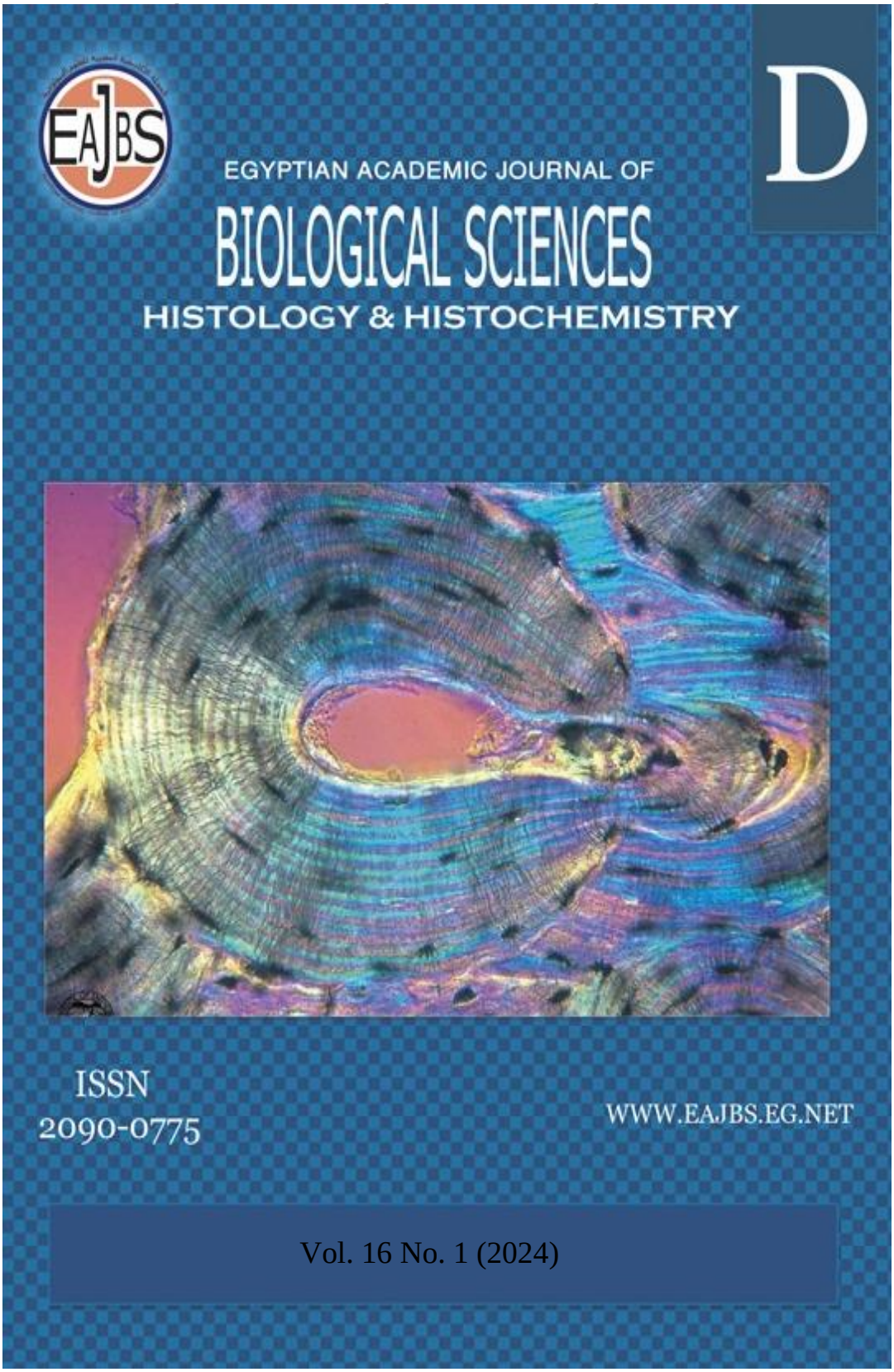

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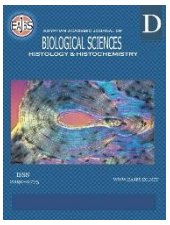

Sexual Dimorphism of Dopaminergic Neurons and Microglia in The Basal Ganglia of Adult Mice

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ABSTRACT

Differences between male and female brains are correlated with sex-specific variation in behaviour and vulnerability to neuropsychiatric diseases. The basal ganglia play a pivotal role in regulating motor and executive functions as well as emotions. Although the basal ganglia contain a high density of receptors for sex hormones, scarce data are available about the sexual dimorphism of cell populations within the basal ganglia. The present study aims to investigate sex-specific differences in two cell populations within the basal ganglia including the dopaminergic neurons and the microglia. Thus, we assessed the optical density of the dopaminergic neurons within the substantia nigra and their projections to the caudate-putamen and globus pallidus by tyrosine hydroxylase immunohistochemistry as well as the density and morphology of IBA-1-expressing microglia in adult (3-months-old) male and female mice. We demonstrated that the substantia nigra contains a higher density of dopaminergic neurons in female mice, with more intensively stained projections in caudate-putamen and globus pallidus. The female substantia nigra and globus pallidus showed an increased number of microglia. Additionally, the microglia exhibited an activated morphology with increased complexity in the female globus pallidus. Our data provide novel anatomical and structural evidence for sex-dependent differences in basal ganglia neuronal circuits and, consequently, the susceptibility to neurological disorders, e.g., Parkinson's disease. This may help a better understanding of the neuropathological diversities and may allow for the design of personalised therapeutic approaches for better treatment outcomes in males and females.

INTRODUCTION

Brain structure and function vary between healthy male and female subjects (Del Mauro *et al.,* 2022). This variability, also known as sexual dimorphism, may underlie the sex-dependent differences in brain functions, e.g., cognition, emotion and social behaviour, under physiological conditions as well as the risk of developing neuro-psychiatric diseases (Meoni *et al.,* 2020, Zalewska *et al.,* 2022, Bianco *et al.,* 2023, Ullah *et al.,* 2019).

Sexual dimorphism has been described in defined brain sub-regions in humans. For instance, females display larger hippocampus and thalamus (Murphy *et al.,* 1996), while the amygdala, subiculum (Gurlek Celik and Tiryaki, 2023) and hypothalamus (Isıklar *et al.,* 2022) have relatively greater volume in males. These sex-specific regional differences may contribute to the receptor density of sex steroid hormones during developmental stages (Witte *et al.,* 2010).

The basal ganglia primarily involve subcortical nuclei that are responsible for the regulation of motor and executive functions in addition to emotions. The caudate nucleus and putamen (caudate-putamen or dorsal striatum) represent key components of the basal ganglia. The dopaminergic neurons in the substantia nigra pars compacta project to the medium-sized spiny neurons in the caudate-putamen via nigrostriatal fibres (Abel and Rissman, 2012), which in turn send output pathways. These outputs include 1. an indirect excitatory striatopallidal pathway (via the globus pallidus that conveys the information to the reticular part of the substantia nigra) mediated by D2 receptors, and 2. a direct inhibitory striatonigral pathway mediated by D1 receptors (Lanciego *et al.,* 2012). The substantia nigra and globus pallidus project to the thalamic and subthalamic nuclei, which in turn project to the motor and sensory cortex. Finally, a corticostriatal input to the striatum forms a closed circuit (Foster *et al.,* 2021, Lanciego *et al.,* 2012). Within the basal ganglia, tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of dopamine, is produced by the substantia nigra dopaminergic neurons that project to the caudate-putamen and the globus pallidus (Abel and Rissman, 2012).

There are few studies investigating sexual dimorphism in the basal ganglia circuit. Recent studies in humans showed that putamen and globus pallidus are larger in males as compared to females (Gurlek Celik and Tiryaki, 2023, Rijpkema *et al.,* 2012, Del Mauro *et al.,* 2022), while caudate nucleus is shown to have a greater size in females (Del Mauro *et al.,* 2022) in addition to an interaction with the ageing process (Wang *et al.,* 2019). However, the sexual dimorphism at the cellular population level has not been elucidated yet.

Dysfunction of the basal ganglia circuit is involved in motor and psychiatric disorders. For instance, Parkinson's disease is manifested by progressive depletion of the dopamine-producing neurons in the substantia nigra and thus, decreased dopamine release by the nigrostriatal pathway in the caudate nucleus and putamen (Hayes, 2019, Ali and Morris, 2015). In addition to dopaminergic neuronal loss, glial cell activation, particularly the microglia, is a crucial contributor to the disease progression (Sanchez-Guajardo *et al.,* 2013). Epidemiological studies demonstrated that men have a higher risk of developing Parkinson's disease, suggesting the involvement of distinct sex-dependent neuropathological pathways in disease development and differential neuroprotective mechanisms (Cerri *et al.,* 2019).

Importantly, sexual dimorphism of specific cell populations that modulate brain circuits may play critical roles in behavioural differences and the risk of disease development (Abel and Rissman, 2012). Thus, in the current study, we observed the sexual dimorphism in two cell populations of the basal ganglia circuit: 1. the dopaminergic neurons (tyrosine hydroxylase-expressing neurons) within the substantia nigra as well as their projections to the caudate-putamen and globus pallidus; and 2. the microglia, which are considered the resident brain immune cells (Villa *et al.,* 2016), and their complexity within the caudate-putamen, globus pallidus and substantia nigra.

As these two cell populations are closely correlated with the development of Parkinson's disease, our findings will

help understand their sexual dimorphism within the basal ganglia loop and, hence, may provide a better understanding of the neuropathological diversities between female and male brains.

MATERIALS AND METHODS

1.Experimental Animals:

Three-month-old adult (30 – 35 g) male and female C57BL/6 mice were used (n = 6 mice per group). All mice were housed in standard cages for two weeks (3 mice of the same gender per cage) under controlled temperature (22 – 23°C), humidity (50 – 55%) and lighting conditions (12 hours of light, 12 hours of dark light on at 7 a.m., and light off at 7 p.m.) to acclimatise and avoid differences due to housing conditions. Food and water were provided ad libitum throughout the experiments. Animal use was approved by the Institutional Animal Care and Use Committee (IACUC), Kafr-Elsheikh University, Egypt (ethical approval number: KFS-IACUC/158/2023). Additionally, the animal experiments were conducted according to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.Tissue Processing:

After two weeks of acclimatisation, mice were sacrificed around the same time of day. Mice were deeply anaesthetized by inhalation of isoflurane until the pinch-paw withdrawal reflex disappeared. The thoracic cavity was open and mice were perfused intracardiacally with 9% NaCl for 5 min, followed by 4% formalin solution for 10 min. Brains were carefully removed from the skull and post-fixed in a 4% formalin for 20 hours, followed by processing for routine histopathological staining using the standard paraffin method. Briefly, brains were subjected to ascending graded ethanol series for dehydration; ethanol was cleared using xylene; and finally, brains were embedded in paraffin wax. Brains were cut into 10 µm-thick sections across the rostro-caudal axis of the brain using a rotatory microtome.

3.Histological and immunohistochemical staining:

The brain sections were deparaffinized using xylene, followed by descending concentrations of ethanol and, finally, rehydrated in distilled water. To investigate the overall morphology, the slices were processed for Nissl staining using cresyl violet acetate 0.2% in acetate buffer. Then, brain sections were dehydrated by immersion in absolute ethanol three times followed by clearing in xylene twice each for 5 min. Then, slides were covered using Entellan (Watson *et al.*, 2010).

For immunohistochemistry, brain slices were incubated with 3% hydrogen peroxide (H2O2) for 1 hour to counteract the endogenous peroxidase activity. This was followed by rinsing using 2% Triton in phosphate-buffered saline (2% PBS-T) three times each for 5 min at room temperature (RT). After that, sections were incubated for 1 hour with 10% goat serum and 1% BSA in 2% PBS-T. Afterwards, sections were incubated with the primary antibodies (anti-tyrosine hydroxylase, 1:400, # ab137869, Abcam, United Kingdom (Magdy *et al.*, 2022); and anti-IBA-1, 1:1000, # ab5076 Abcam, United Kingdom (Ijaz *et al.*, 2022)) diluted in the blocking solution overnight (16 hours) at 4°C.

Sections were rinsed with 2% PBS-T three times each for 5 min, then incubated with the biotin-conjugated secondary antibodies (IgG) diluted in blocking solution for 1 hour at RT. Sections were subsequently washed using 2% PBS-T three times each for 5 min at RT and incubated with an avidin-biotin complex diluted in 2% PBS-T for 1 hour at RT. Then, the slides were rinsed using 2% PBS-T. Finally, sections were incubated with diaminobenzidine to develop the chromogenic reaction, followed by multiple rinsing steps in PBS. Sections were left to dry and then covered with Entellan (Hernández-Pérez *et al.*, 2019).

4.Image Acquisition and Analysis:

The acquisition of photomicrographs of immunohistochemically stained brain sections was done using the bright field mode of an Olympus® CX41 microscope equipped with an Olympus® SC100 camera using an x40 objective. The camera settings were kept identical during image acquisition and processing in one experimental set to avoid bias. The experimental groups were obscured from the investigator. Equivalent fields in sections including parallel substantia nigra, caudate-putamen and globus pallidus from all groups were processed for the assessment using ImageJ software (Bethesda, MD, United States). The optical density (OD) of the tyrosine hydroxylase (TOH)-positive cells and fibres in substantia nigra compacta (SNc) and substantia nigra reticularis (SNr), respectively, was assessed by calculation of the mean grey value after subtraction from the background staining and expressed as arbitrary units (A.U.) (Magdy *et al.,* 2022). The number of IBA-1-positive microglia was evaluated using the multipoint tool of the measurement menu of image J in a delineated area of 240 µm x 240 µm, then neutralised to mm^2 to be expressed as cell density (positive cells/mm^2). A minimum of six independent fields were analysed in each brain region (Schindelin *et al.,* 2012).

To analyse the complexity and density of the glial processes, the pseudocolor system in image J was used. Briefly, images were converted to 8-bit mode and processed using lookup tables (LUT) and the 6 shades option after adjusting the contrast. The colour within the image reflects the main grey value and subsequently, the staining intensity was assessed as follows: red: very strong, green: strong, dark blue: moderate, light blue: weak, magenta: very weak, yellow-white: no expression (Schindelin *et al.,* 2012).

5.Statistical analysis:

Data were analysed by Graph-Pad Prism software. The non-parametric Mann-Whitney-U-test was used to compare the two experimental groups. The values were shown as mean ± standard error of the mean (SEM) and were considered as statistically different when $P < 0.05$.

RESULTS

1-Histological Architecture of Basal Ganglia:

We used Nissl staining via the cresyl violet method to demonstrate differences in the histological architecture between males and females. We didn't observe major variations in the morphology or cellular architecture of the substantia nigra (Fig. 1a,b), caudate-putamen (Fig. 1c,d), or globus pallidus (Fig. 1e,f).

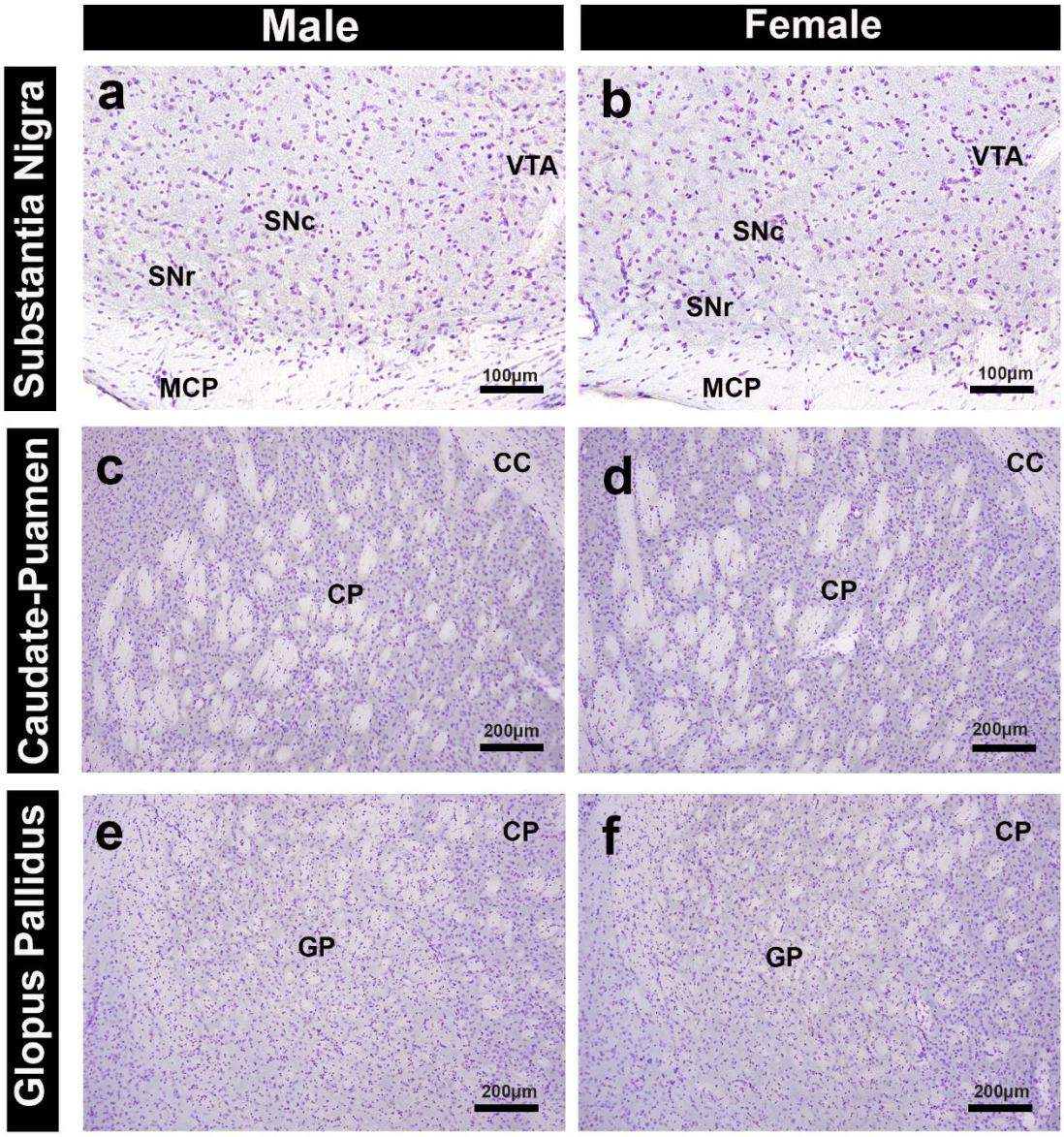

Fig. 1. Morphological and histological architecture of the basal ganglia. a) Representative photomicrograph showing Nissl-stained (cresyl violet) brain section from the substantia nigra of male and b) female mice showing similar morphology and cellular distribution (nuclei: dark violet, cytoplasm: bright violet) in both sexes. c) Representative photomicrograph showing Nissl-stained (cresyl violet) sections from caudate-putamen of male and d) female mice showing comparable morphology and cellular distribution in both sexes. e) Representative photomicrograph showing Nissl-stained (cresyl violet) globus pallidus of male and f) female mice showing no differences in morphology and cellular distribution. VTA: ventral tegmental area; MCP: middle cerebellar peduncle; SNr: substantia nigra pars reticularis; SNc: substantia nigra pars compacta; CC: corpus callosum; CP: caudate-putamen; GP: globus pallidus.

2-Dopaminergic Tyrosine Hydroxylase-Positive Neurons and Fibres in the Substantia Nigra:

The tyrosine hydroxylase was used as a marker of the dopaminergic neurons and fibres. Within the substania nigra, strongly stained dopaminergic fibres in the pars reticularis as well as positively stained, densely packed dopaminergic neurons in the pars compacta have been demonstrated in both sexes. In female mice, the optical density of the tyrosine hydroxylase-positive fibres within the substantia nigra pars reticularis was significantly increased in female mice (69.1±3.2) than in male mice (45.6±4.4) (P = 0.03) (Fig. 2a,b,c). Consistently, the optical density

of tyrosine hydroxylase-positive dopaminergic neurons within the substantia nigra pars compacta in female mice (113.5±2.2) was more pronounced than in male mice (101.9±4.8) (P = 0.04) (Fig. 2a,b,d). In addition, we observed some scattered tyrosine hydroxylase-positive dopaminergic neurons within the substantia nigra pars reticularis in female mice more frequently than in male mice.

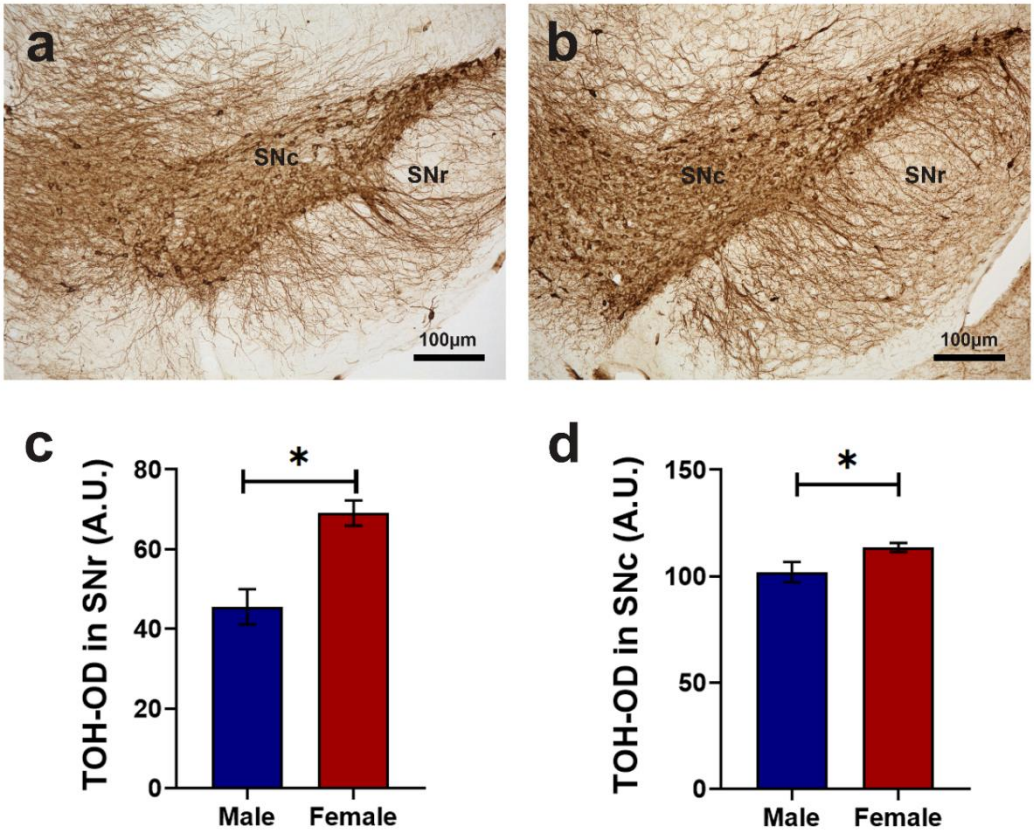

Fig. 2. Dopaminergic tyrosine hydroxylase-positive neurons and fibres in the substantia nigra. a) Representative photomicrograph of brain section showing the substantia nigra pars reticularis (SNr) and substantia nigra pars compacta (SNc) stained against tyrosine hydroxylase (brown colour) from male mice and b) female mice. c) Quantitative analysis of the optical density (OD) of tyrosine hydroxylase-positive fibres in substantia nigra pars reticularis expressed as arbitrary units (A.U.). d) Quantitative analysis of the optical density of tyrosine hydroxylase-positive fibres and neurons in substantia nigra pars compacta. *: $P < 0.05$ using the non-parametric Mann-Whitney-U-test. Values are expressed as mean ± SEM (standard error of the mean).

3-Dopaminergic Tyrosine Hydroxylase-Positive Fibres in Caudate-Putamen and Globus Pallidus:

In both sexes, the caudate-putamen and globus pallidus showed strong positive staining, which was more evident in the caudate-putamen as compared to the globus pallidus. A dense interlacing tyrosine hydroxylase-positive fibre network was detected within the caudate-putamen, while in globus pallidus, the tyrosine hydroxylase-positive fibres showed a scattered appearance. In male mice, the optical density of tyrosine hydroxylase-positive fibres within the caudate-putamen (90.4±1.2) (Fig. 3a,b,g) was significantly less than in the female mice (116.5±1.7) (P = 0.004) (Fig. 3d,e,g). Similarly, the optical density of tyrosine hydroxylase positive fibres within the globus pallidus was significantly lower in male mice (33.3±0.9) (Fig. 3a,c,h) than in female mice (49.3±0.6) (P = 0.004) (Fig. 3d,f,h).

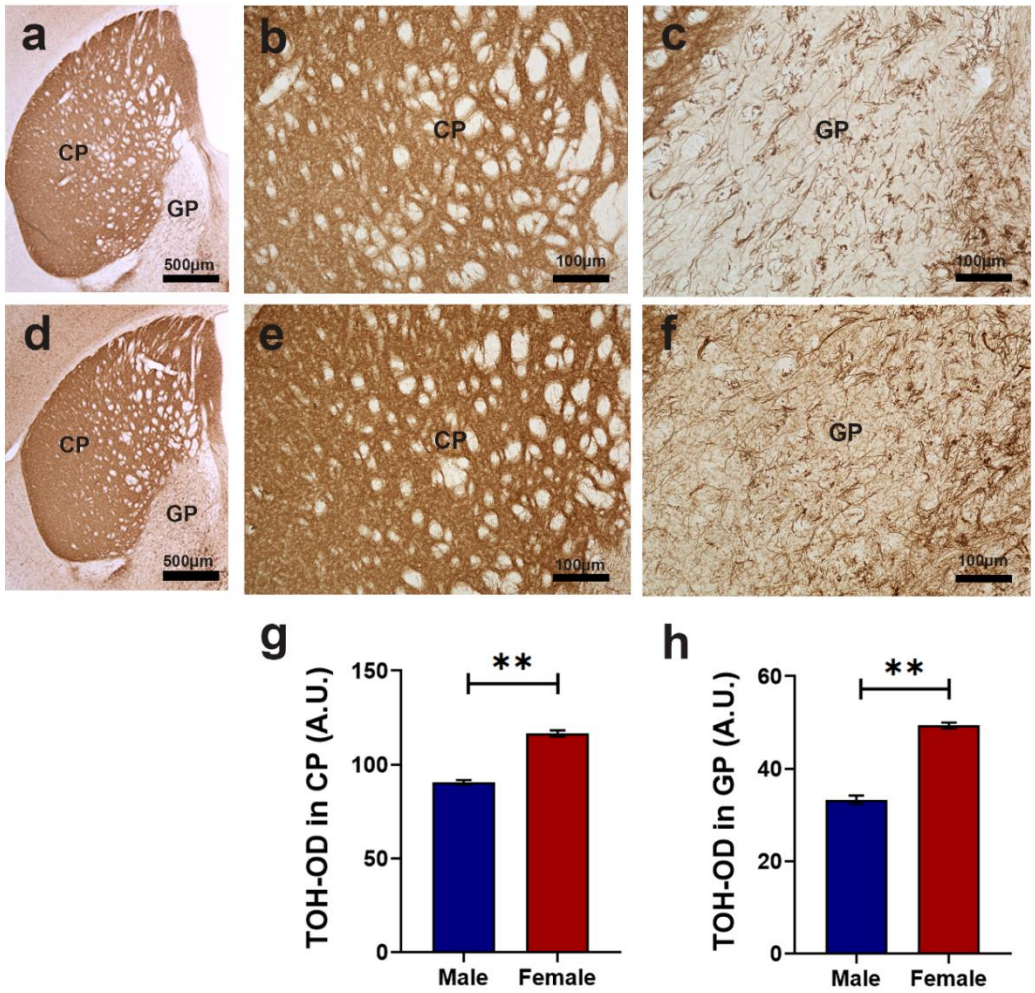

Fig. 3. Dopaminergic tyrosine hydroxylase-positive fibres in caudate-putamen and globus pallidus. a) Low-magnification representative photomicrograph of brain section showing the caudate-putamen (CP) and globus pallidus (GP) in male mice stained against tyrosine hydroxylase (brown colour). b) High-magnification representative photomicrograph showing dopaminergic tyrosine hydroxylase-positive dense fibres (brown) within caudate-putamen in male mice. c) High-magnification representative photomicrograph showing dopaminergic tyrosine hydroxylase-positive scattered fibres (brown) within globus pallidus in male mice brain. d) Low-magnification representative photomicrograph of tyrosine hydroxylase-stained brain section showing the caudate-putamen and globus pallidus in female mice. e) High-magnification representative photomicrograph showing dopaminergic tyrosine hydroxylase-positive dense fibres (brown) within the caudate-putamen in female mice. f) High-magnification representative photomicrograph showing dopaminergic tyrosine hydroxylase-positive scattered fibres (brown) within globus pallidus in the brain of female mice. g) Quantitative analysis of the optical density (OD) of tyrosine hydroxylase-positive fibres in caudate-putamen expressed as arbitrary units (A.U.). h) Quantitative analysis of the optical density of tyrosine hydroxylase-positive fibres in the globus pallidus. **: $P < 0.01$ using the non-parametric Mann-Whitney-U-test. Values are expressed as mean ± SEM.

4-Microglia Density in The Substantia Nigra:

To visualise the microglia and their network, IBA-1 staining was used. The substantia nigra in both male and female mice demonstrated a network of microglial cells, as indicated by IBA-1 staining. There was no significant difference in microglial cell density between substantia nigra pars compacta

and pars reticularis; therefore, the values from both subregions were pooled together. The number of IBA-1+ cells in the substantia nigra was significantly higher in female mice (615.7±23 cells/mm^2) as compared to male mice (483.3±33.2 cells/mm^2) (P = 0.02) (Fig. 4a,b,c).

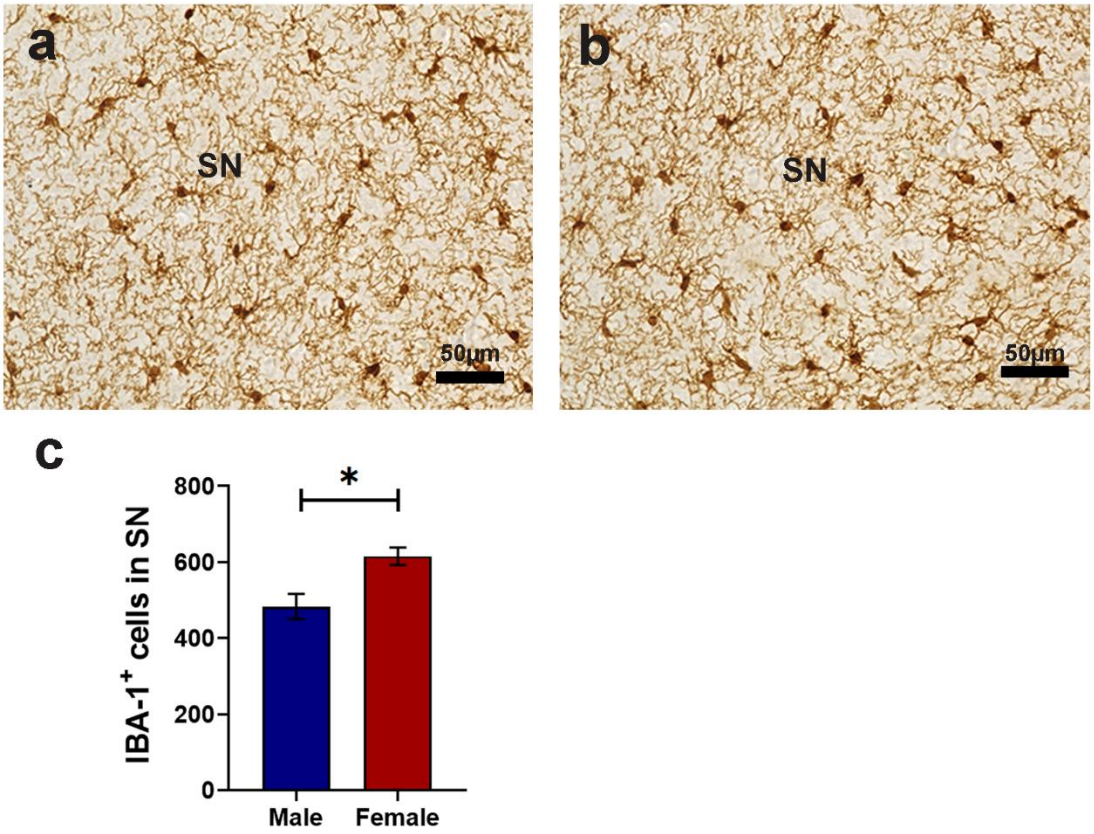

Fig. 4. Microglia density in the substantia nigra. a) Representative photomicrograph of brain section showing the substantia nigra (SN) in male mice and b) in female mice stained against microglial marker IBA-1 (brown). c) Quantitative analysis of the IBA-positive (+) microglia in substantia nigra expressed as cells/mm^2. *: $P < 0.05$ using the non-parametric Mann-Whitney-U-test. Values are expressed as mean ± SEM.

5-Microglia Density in Caudate-Putamen and Globus Pallidus:

The mouse caudate-putamen and globus pallidus showed a network of microglial cells, as indicated by the strong expression of IBA-1. The number of IBA-1+ cells was not significantly different in female mice (305.9±9.3 cells/mm^2) as compared to male mice (317.5±13 cells/mm^2) (P = 0.6) in the caudate-putamen (Fig. 5a,b,e). The globus pallidus seemed to involve higher microglial density in both sexes as compared to caudate-putamen. However, the IBA-1+ cell density in the globus pallidus was significantly less in male mice (476.3±29.2 cells/mm^2) as compared to female mice (584.7±33.6 cells/mm^2) (P = 0.049) (Fig. 5c,d,f).

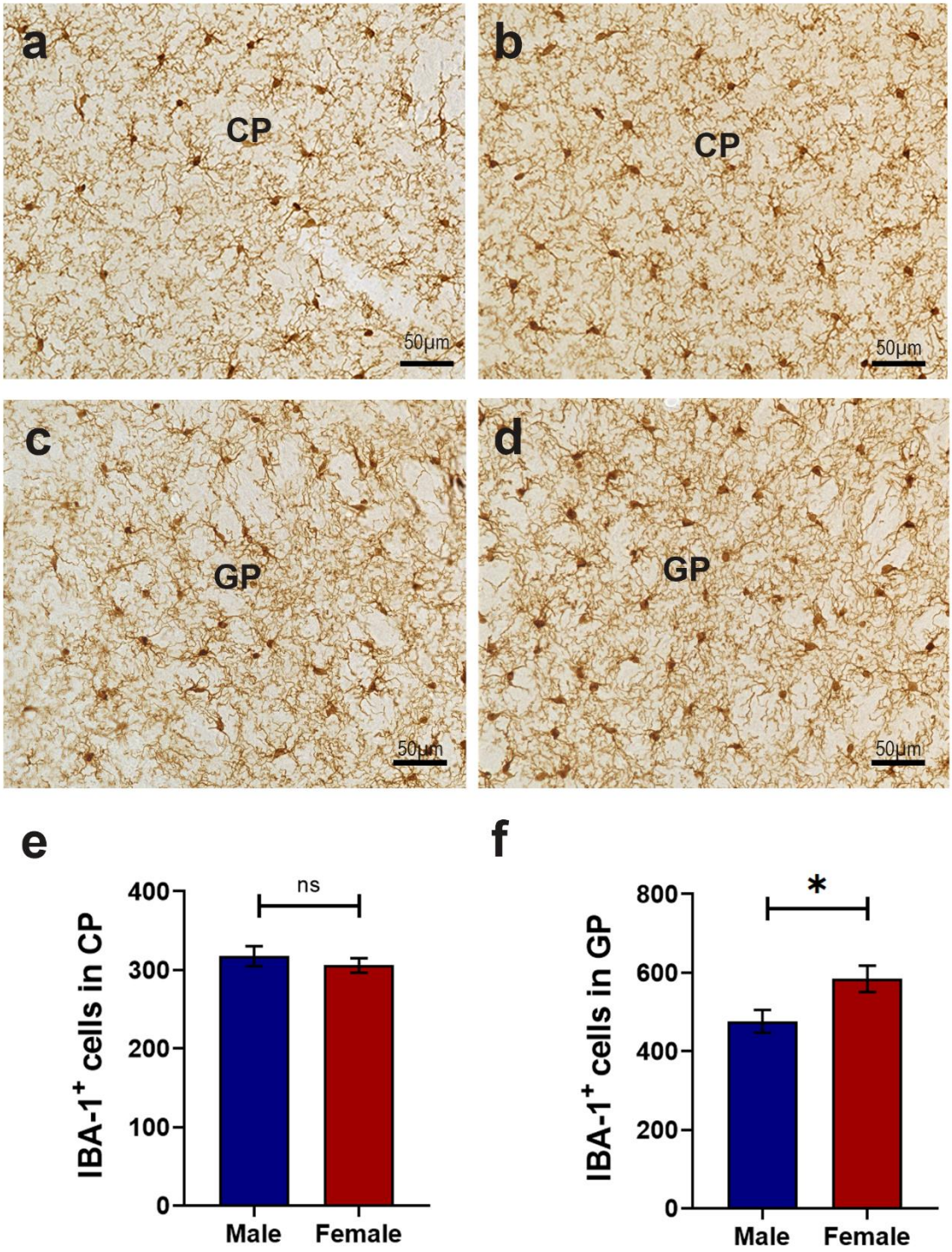

Fig. 5. Microglia density in caudate-putamen and globus pallidus. a) Representative photomicrograph of brain section showing the caudate-putamen (CP) in male mice and b) in female mice stained against microglial marker IBA-1 (brown). c) Representative photomicrograph of brain section showing the globus pallidus (GP) in male mice and d) in female mice stained against microglial marker IBA-1 (brown). e) Quantitative analysis of the IBA-positive (+) microglia in caudate-putamen expressed as cells/mm^2. f) Quantitative analysis of the IBA-positive (+) microglia in globus pallidus expressed as cells/mm^2. n.s.: non-significant; *: $P < 0.05$ using the non-parametric Mann-Whitney-U-test. Values are expressed as mean ± SEM.

6-Microglia Morphology in The Basal Ganglia:

To analyse the morphological complexity of microglia, we processed the photomicrographs showing IBA-1 staining with the pseudocolour system of image J. The substantia nigra showed generally complex microglial branching.

Nevertheless, we didn't find significant differences in the branching complexity of microglial processes within the substantia nigra between male and female mice (Fig. 6a,b). Furthermore, we didn't observe significant differences in the soma size or branching complexity of microglial processes within the caudate-putamen between male and female mice (**Fig. 6c,d**). The globus pallidus seemed to involve a more complex microglial network, particularly in females, as compared to the caudate-putamen. In addition, the microglia in female mice within the globus pallidus appeared to display a larger soma and more pronounced branching complexity with longer and thicker processes as compared to male mice (Fig. 6e,f).

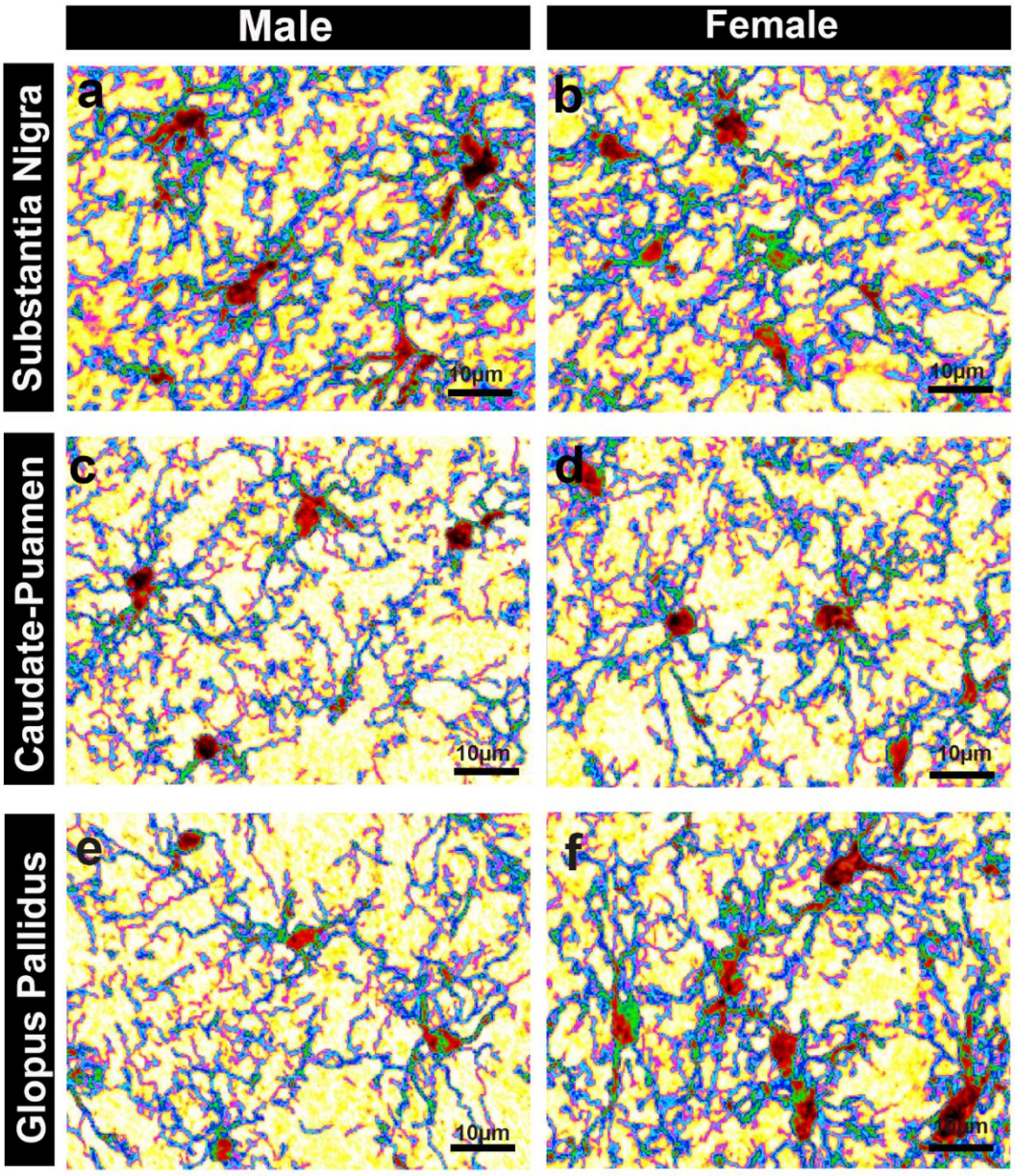

Fig. 6. Microglia network complexity within the basal ganglia based on lookup tables (LUT) 6 shades mode in ImageJ. a) Representative high-magnification pseudocolour images from the substantia nigra of male and b) female mice showing high, but similar, branching complexity of microglial in both sexes. c) Representative high-magnification pseudocolour images from the caudate-putamen of male and d) female mice showing comparable complex microglial branching in both sexes. e) Representative high-magnification pseudocolour images from the globus pallidus of male and f) female mice showing more complex microglial branching and a bigger soma in female mice. IBA-1 staining: Red: very strong; green: strong; dark blue: moderate; light blue: weak; magenta: very weak; yellow-white: no staining.

DISCUSSION

The current study provides for the first-time evidence of sexual dimorphism in two cell populations of the basal ganglia circuit: the dopaminergic neurons and their projections in addition to the microglia.

Here, we showed that the dopaminergic neurons in the substantia nigra in addition to the dopaminergic innervation of caudate-putamen and globus pallidus, as indicated by the tyrosine hydroxylase immunoreaction, are of higher density in female mice compared to male mice. Importantly, oestrogen seems to play a crucial role in tyrosine hydroxylase biosynthesis (Ma et al., 2007). This regulatory effect of oestrogen is sex-specific, as it inhibits the activity of the tyrosine hydroxylase promoter in males while enhances the transcription of the tyrosine hydroxylase gene in females (Thanky *et al.,* 2002). Oestrogen is also thought to mediate neuronal integrity and induce functional modulation of the dopaminergic system (Ma *et al.*, 2007). Noteworthy, the sexual dimorphism in the midbrain dopaminergic neuronal differentiation may also be directly regulated by the gonadal chromosome (Kopsida *et al.*, 2009, Carruth *et al.,* 2002). In line with our data and with these findings, the substantia nigra of female rats displayed a greater number of tyrosine hydroxylase-positive cells (Ma *et al.*, 2007). Consistently, similar observations were reported in other brain regions. For instance, locus coeruleus has more tyrosine hydroxylase-positive neurons in female mice as compared to male mice due to the regulatory effects induced by oestrogen receptor beta (Pendergast *et al.*, 2008). A similar finding has been reported in the medial preoptic area, where the dopaminergic neurons were fewer in male mice, however, independently of androgen-Bax related apoptosis (Forger *et al.,* 2004) but rather via modulation of the oestrogen receptor (Bodo and Rissman, 2008) and suppression of caspase-dependent cell death (Waters and Simerly, 2009) in female mice.

In contrast, the number of tyrosine hydroxylase-positive neurons in the ventral tegmental area didn't show sex-dependent variability along the rostrocaudal extent, although their activation or inhibition affects the behaviour differently in male than in female mice indicating, however, functional sexual dimorphism (Dunigan *et al.*, 2021). In addition, the dopaminergic projections from the ventral tegmental area to the forebrain targets including basolateral amygdala, a dopaminergic circuit that contributes to motivational and emotional behaviour, didn't show sex-related differences in dopaminergic axon density, however, the synaptic buttons, where dopamine is released, are more frequent in males (Manion *et al.*, 2022). Thus, the sexual dimorphism in the dopaminergic neurons and their projections may be region and circuit-specific. Taken all together, the higher number of tyrosine hydroxylase-positive neurons reported in multiple brain regions as well as observed results in our study may play a protective role in females against Parkinson's disease.

The glial cells, particularly the resident microglia, represent an essential mediator for brain immunity and homeostasis (Sanchez-Guajardo *et al.*, 2013, Schwarz *et al.*, 2012). Sexual dimorphism of microglial cells may explain differences between males and females in neuroinflammatory reactions in response to brain injury (Villapol *et al.*, 2017) as well as in vulnerability to progression of neurodegenerative diseases including Parkinson's disease (Guillot-Sestier *et al.,* 2021).

Thus, we investigated the sex-dependant differences in microglial number and complexity in basal ganglia. Our data showed higher microglial density in the substantia nigra and globus pallidus in addition to a more complex branching pattern in the globus pallidus of the female than in male mice. This is in line with previous reports that showed

sex-specific differences in microglia. Interestingly, microglia exhibit spatiotemporal heterogeneity among various brain regions between males and females (Lenz and McCarthy, 2014). The microglial cell density was higher in female mice during development and then showed a significant increase in adulthood males in the cerebral cortex, hippocampus and amygdala, while the cerebellar microglia didn't show these differences (Lenz and McCarthy, 2014, Guneykaya *et al.,* 2018). Another study showed contrasting findings in rat brains as the microglia in the parietal cortex, hippocampal subregions, amygdala and paraventricular nucleus of males were more numerous during the early postnatal development period while females' microglia in the parietal cortex, hippocampal subregions, and amygdala significantly increased during early adulthood (2 months) (Schwarz *et al.,* 2012). This sex-mediated effect could be due to microglial expression of oestrogen and testosterone receptors; nevertheless, the expression pattern differs across brain regions (O'Connor and Nissen, 2023, Villa *et al.,* 2016).

The hippocampal microglia show increased expression of mature phenotype genes in female mice compared to age-matched males, suggesting a sexual dimorphism in maturation and immunoreactivity (Hanamsagar *et al.,* 2017). This is also consistent with the significant differences in the transcriptomic profile of hippocampal and cortical microglia between males and females (Guneykaya *et al.,* 2018). Recently, it has been widely accepted that two microglial phenotypes based on their gene expression profile exist, M1 microglia, which induce pro-inflammatory responses including tumour necrosis alpha (TNFα), interleukins IL-6 and IL-1ß; and M2 microglia that induce an anti-inflammatory response by IL-10 (Crain *et al.,* 2013, Mohamad et al., 2019, O'Connor and Nissen, 2023). Interestingly, the female mice show a predominance of M1 microglia while M2 microglia are more predominant in male mice (Crain *et al.,* 2013). In addition, microglial functional properties showed sexual dimorphism. For instance, the antigen-presenting capacity, which is essential for the recognition of damage-associated molecules or pathogens (O'Connor and Nissen, 2023), was upregulated in the cortex and hippocampus of male mice (Guneykaya *et al.,* 2018). This observation may indicate an association between microglial density and the antigen-presenting potential and thus, a more efficient maintenance of homeostasis during inflammation.

The state of microglial activity could be correlated with the microglial morphology (Schwarz *et al.,* 2012, Vidal-Itriago *et al.,* 2022, Leyh *et al.,* 2021, Kongsui *et al.,* 2014). It was reported that the microglia in female mice exhibited more morphological features of activated phenotype than in male mice (Vidal-Itriago *et al.,* 2022). This is in accordance with a previous study demonstrating that microglia in the parietal cortex, hippocampus and amygdala showed an activated phenotype in female rats as indicated by numerous long and thick processes (Schwarz *et al.,* 2012). Taken all together, the higher microglial density and the complex morphology within the basal ganglia in female mice could differently modulate the neuronal sensitivity that predisposes to neurodegeneration and therefore, may be correlated to resilience to develop Parkinson's disease (Howell *et al.,* 2020).

In conclusion, our data provides novel evidence of variation in cell populations including dopaminergic neurons and microglia within the basal ganglia between male and female mice and emphasizes anatomical and structural sex-dependent differences in basal ganglia neuronal circuits and consequently, the susceptibility to neurological disorders e.g., Parkinson's disease. It is still to be elucidated which molecular and signalling pathways are

underlying this sexual dimorphism. Identifying such mechanisms will allow the designing of personalised therapeutic approaches for better treatment outcomes in males and females.

Conflict of Interest: the authors declare no conflict of interest

REFERENCES

Abel, J. L. & Rissman, E. F. (2012). Location, location, location: genetic regulation of neural sex differences. *Reviews in Endocrinal and Metabolic Disorders,* 13, 151-61.

Ali, K., & Morris, H. R. (2015). Parkinson's disease: chameleons and mimics. *Practical Neurology,* 15, 14-25.

Bianco, A., Antonacci, Y. & Liguori, M. (2023). Sex and Gender Differences in Neurodegenerative Diseases: Challenges for Therapeutic Opportunities. *International Journal of Molecular Sciences,* 24, 6354.

Bodo, C. & Rissman, E. F. (2008). The androgen receptor is selectively involved in organization of sexually dimorphic social behaviors in mice. *Endocrinology,* 149, 4142-50.

Carruth, L. L., Reisert, I. & Arnold, A. P. (2002). Sex chromosome genes directly affect brain sexual differentiation. *Nature Neuroscience,* 5, 933-4.

Cerri, S., Mus, L. & Blandini, F. (2019). Parkinson's Disease in Women and Men: What's the Difference? *Journal of Parkinsons Disease,* 9, 501-515.

Crain, J. M., Nikodemova, M. & Watters, J. J. (2013). Microglia express distinct M1 and M2 phenotypic markers in the postnatal and adult central nervous system in male and female mice. *Journal of Neuroscience Research,* 91, 1143-1151.

Del Mauro, G., Del Maschio, N., Sulpizio, S., Fedeli, D., Perani, D. & Abutalebi, J. (2022). Investigating sexual dimorphism in human brain structure by combining multiple indexes of brain morphology and source-based morphometry. *Brain Structure and Function,* 227, 11-21.

Dunigan, A. I., Olson, D. P. & Roseberry, A. G. (2021). VTA MC3R neurons control feeding in an activity- and sex-dependent manner in mice. *Neuropharmacology,* 197, 108746.

Forger, N. G., Rosen, G. J., Waters, E. M., Jacob, D., Simerly, R. B. & De Vries, G. J. (2004). Deletion of Bax eliminates sex differences in the mouse forebrain. *Proceedings of the National Academy of Sciences U S A,* 101, 13666-71.

Foster, N. N., Barry, J., Korobkova, L., Garcia, L., Gao, L., Becerra, M., Sherafat, Y., Peng, B., Li, X., Choi, J. H., Gou, L., Zingg, B., Azam, S., Lo, D., Khanjani, N., Zhang, B., Stanis, J., Bowman, I., Cotter, K., Cao, C., Yamashita, S., Tugangui, A., Li, A., Jiang, T., Jia, X., Feng, Z., Aquino, S., Mun, H. S., Zhu, M., Santarelli, A., Benavidez, N. L., Song, M., Dan, G., Fayzullina, M., Ustrell, S., Boesen, T., Johnson, D. L., Xu, H., Bienkowski, M. S., Yang, X. W., Gong, H., Levine, M. S., Wickersham, I., Luo, Q., Hahn, J. D., Lim, B. K., Zhang, L. I., Cepeda, C., Hintiryan, H. & Dong, H. W. (2021). The mouse cortico-basal ganglia-thalamic network. *Nature*, 598, 188-194.

Guillot-Sestier, M.-V., Araiz, A. R., Mela, V., Gaban, A. S., O'neill, E., Joshi, L., Chouchani, E. T., Mills, E. L. & Lynch, M. A. (2021). Microglial metabolism is a pivotal factor in sexual dimorphism in Alzheimer's

disease. *Communications Biology*, 4, 711.

Guneykaya, D., Ivanov, A., Hernandez, D. P., Haage, V., Wojtas, B., Meyer, N., Maricos, M., Jordan, P., Buonfiglioli, A., Gielniewski, B., Ochocka, N., Cömert, C., Friedrich, C., Artiles, L. S., Kaminska, B., Mertins, P., Beule, D., Kettenmann, H. & Wolf, S. A. (2018). Transcriptional and Translational Differences of Microglia from Male and Female Brains. *Cell Reports*, 24, 2773-2783.e6.

Gurlek Celik, N. & Tiryaki, S. (2023). Changes in the volumes and asymmetry of subcortical structures in healthy individuals according to gender. *Anatomical Sciences International*, 98, 506-519.

Hanamsagar, R., Alter, M. D., Block, C. S., Sullivan, H., Bolton, J. L. & Bilbo, S. D. (2017). Generation of a microglial developmental index in mice and in humans reveals a sex difference in maturation and immune reactivity. *Glia*, 65, 1504-1520.

Hayes, M. T. (2019). Parkinson's Disease and Parkinsonism. *American Journal of Medicine*, 132, 802-807.

Hernández-Pérez, O. R., Hernández, V. S., Nava-Kopp, A. T., Barrio, R. A., Seifi, M., Swinny, J. D., Eiden, L. E., & Zhang, L. (2019). A Synaptically Connected Hypothalamic Magnocellular Vasopressin-Locus Coeruleus Neuronal Circuit and Its Plasticity in Response to Emotional and Physiological Stress. *Frontiers of Neuroscience*, 13, 196.

Howell, R. D., Dominguez-Lopez, S., Ocañas, S. R., Freeman, W. M. & Beckstead, M. J. (2020). Female mice are resilient to age-related decline of substantia nigra dopamine neuron firing parameters. *Neurobiology of Aging*, 95, 195-204.

Ijaz, L., Nijsure, M., & Fossati, V. (2022). Human Pluripotent Stem Cell Differentiation to Microglia. *Methods in Molecular Biology*, 2454, 455-471.

Isıklar, S., Turan Ozdemir, S., Ozkaya, G. & Ozpar, R. (2022). Hypothalamic volume and asymmetry in the pediatric population: a retrospective MRI study. *Brain Structue and Function*, 227, 2489-2501.

Kongsui, R., Beynon, S. B., Johnson, S. J. & Walker, F. R. (2014). Quantitative assessment of microglial morphology and density reveals remarkable consistency in the distribution and morphology of cells within the healthy prefrontal cortex of the rat. *Journal of Neuroinflammation*, 11, 182.

Kopsida, E., Stergiakouli, E., Lynn, P. M., Wilkinson, L. S. & Davies, W. (2009). The Role of the Y Chromosome in Brain Function. *Open Neuroendocrinology Journal*, 2, 20-30.

Lanciego, J. L., Luquin, N. & Obeso, J. A. (2012). Functional neuroanatomy of the basal ganglia. *Cold Spring Harbor Perspectives in Medicine*, 2, a009621.

Lenz, K. M. & McCarthy, M. M. (2014). A Starring Role for Microglia in Brain Sex Differences. *The Neuroscientist*, 21, 306-321.

Leyh, J., Paeschke, S., Mages, B., Michalski, D., Nowicki, M., Bechmann, I. & Winter, K. (2021). Classification of Microglial Morphological Phenotypes Using Machine Learning. *Frontiers in Cellular Neuroscience*, 15, 701673.

Ma, Y. Y., Kong, S. Z., Yang, L. J., Meng, J. L., Lv, L. C. & He, M. (2007). Sexual dimorphisms of dopaminergic neurons in rat

substantia nigra. *Sheng Li Xue Bao*, 59, 753-8.

Magdy, A., Farrag, E. A. E., Hamed, S. M., Abdallah, Z., El Nashar, E. M., Alghamdi, M. A., Ali, A. A. H., & Abd El-kader, M. (2022). Neuroprotective and therapeutic effects of calcitriol in rotenone-induced Parkinson's disease rat model. *Frontiers in Cellular Neuroscience*, 16, 967813.

Manion, M. T. C., Glasper, E. R. & Wang, K. H. (2022). A sex difference in mouse dopaminergic projections from the midbrain to basolateral amygdala. *Biological Sex Differences*, 13, 75.

Meoni, S., Macerollo, A. & Moro, E. (2020). Sex differences in movement disorders. *Nature Reviews in Neurology*, 16, 84-96.

Mohamad, N.-V., Wong, S. K., Wan Hasan, W. N., Jolly, J. J., Nur-Farhana, M. F., Ima-Nirwana, S. & Chin, K.-Y. (2019). The relationship between circulating testosterone and inflammatory cytokines in men. *The Aging Male*, 22, 129-140.

Murphy, D. G., Decarli, C., Mcintosh, A. R., Daly, E., Mentis, M. J., Pietrini, P., Szczepanik, J., Schapiro, M. B., Grady, C. L., Horwitz, B. & Rapoport, S. I. (1996). Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. *Archive of Gen Psychiatry*, 53, 585-94.

O'Connor, J. L. & Nissen, J. C. (2023). The Pathological Activation of Microglia Is Modulated by Sexually Dimorphic Pathways. *International Journal of Molecular Sciences*, 24(5): 4739.

Pendergast, J. S., Tuesta, L. M. & Bethea, J. R. (2008). Oestrogen receptor beta contributes to the transient sex difference in tyrosine hydroxylase expression in the mouse locus coeruleus. *Journal of Neuroendocrinology*, 20, 1155-64.

Rijpkema, M., Everaerd, D., Van Der Pol, C., Franke, B., Tendolkar, I. & Fernández, G. (2012). Normal sexual dimorphism in the human basal ganglia. *Human Brain Mapping*, 33, 1246-52.

Sanchez-Guajardo, V., Barnum, C. J., Tansey, M. G. & Romero-Ramos, M. (2013). Neuroimmunological processes in Parkinson's disease and their relation to α-synuclein: microglia as the referee between neuronal processes and peripheral immunity. *ASN Neurology*, 5, 113-39.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682.

Schwarz, J. M., Sholar, P. W. & Bilbo, S. D. (2012). Sex differences in microglial colonization of the developing rat brain. *Journal of Neurochemistry*, 120, 948-63.

Thanky, N. R., Son, J. H. & Herbison, A. E. (2002). Sex differences in the regulation of tyrosine hydroxylase gene transcription by estrogen in the locus coeruleus of TH9-LacZ transgenic mice. *Brain Research and Molecular Brain Research*, 104, 220-6.

Ullah, M. F., Ahmad, A., Bhat, S. H., Abu-Duhier, F. M., Barreto, G. E. & Ashraf, G. M. (2019). Impact of sex differences and gender specificity on behavioral characteristics and pathophysiology of neurodegenerative disorders.

Neuroscience & Biobehavioral Reviews, 102, 95-105.

Vidal-Itriago, A., Radford, R. A. W., Aramideh, J. A., Maurel, C., Scherer, N. M., Don, E. K., Lee, A., Chung, R. S., Graeber, M. B. & Morsch, M. (2022). Microglia morphophysiological diversity and its implications for the CNS. *Frontiers in Immunology*, 13, 997786.

Villa, A., Vegeto, E., Poletti, A. & Maggi, A. (2016). Estrogens, Neuroinflammation, and Neurodegeneration . *Endocrinology Reviews*, 37, 372-402.

Villapol, S., Loane, D. J. & Burns, M. P. (2017). Sexual dimorphism in the inflammatory response to traumatic brain injury. *Glia*, 65, 1423-1438.

Wang, Y., Xu, Q., Luo, J., Hu, M. & Zuo, C. (2019). Effects of Age and Sex on Subcortical Volumes. *Frontiers of Aging Neuroscience*, 11, 259.

Waters, E. M. & Simerly, R. B. (2009). Estrogen induces caspase-dependent cell death during hypothalamic development. *Journal of Neuroscience*, 29, 9714-8.

Watson, C., Kirkcaldie, M., & Paxinos, G. (2010). Chapter 11 - Techniques for studying the brain. In Charles Watson, Matthew Kirkcaldie, & George Paxinos (Eds.), San Diego: Academic Press. *The Brain*, 153-165.

Witte, A. V., Savli, M., Holik, A., Kasper, S. & Lanzenberger, R. (2010). Regional sex differences in grey matter volume are associated with sex hormones in the young adult human brain. *Neuroimage*, 49, 1205-12.

Zalewska, T., Pawelec, P., Ziabska, K. & Ziemka-Nalecz, M. (2022). Sexual Dimorphism in Neurodegenerative Diseases and in Brain Ischemia. *Biomolecules*, 13(1): 26.

ARABIC SUMMARY

إزدواج الشكل الجنسي في العقد القاعدية للفئران البالغة

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ترتبط الإختلافات الموجودة بين أدمغة كلاً من الذكور والإناث بالإختلافات المرتبطة بالجنس في السلوك والقابلية للإصابة بالأمراض العصبية والنفسية. تلعب العقد القاعدية دورًا محوريًا في تنظيم الوظائف الحركية والتنفيذية والعواطف. على الرغم من أن العقد القاعدية تحتوي على كثافة عالية من مستقبلات الهرمونات الجنسية، إلا أن البيانات المتاحة حول إزدواج الشكل الجنسي لمجموعات الخلايا داخل العقد القاعدية نادرة جداً. تهدف الدراسة الحالية إلى دراسة الإختلافات المرتبطة بالجنس في مجموعتين من الخلايا داخل العقد القاعدية بما في ذلك الخلايا العصبية الدوباميني والخلايا الدبقية الصغيرة. بالإضافة إلى ذلك، قمنا بتقييم الكثافة الضوئية للخلايا العصبية الدوباميني داخل المادة السوداء وإمدادتها إلى البطامة- النواة المذنبة والكرة الشاحبة بواسطة تقنية الكيمياء المناعية للكشف عن هيدروكسيلاز التيروسين وكذلك الكثافة والشكل الظاهري ل IBA-1 و الذي يعبر عن الخلايا الدبقية الصغيرة. لقد أثبتنا أن المادة السوداء تحتوي على كثافة أعلى من الخلايا العصبية الدوباميني في إناث الفئران مع إمدادات مصبوغة بشكل مكثف في البطامة- النواة المذنبة والكرة الشاحبة. كما أظهرت المادة السوداء والكرة الشاحبة في الإناث عددًا متزايدًا من الخلايا الدبقية الصغيرة. بالإضافة إلى ذلك، أظهرت الخلايا الدبقية الصغيرة شكلًا ظاهرياً نشطًا مع زيادة التعقيد في الكرة الشاحبة للإناث. توفر نتائجنا أدلة تشريحية وتركيبية جديدة للإختلافات المرتبطة بالجنس في الدوائر العصبية للعقد القاعدية، وبالتالي القابلية للإصابة بالإضطرابات العصبية مثل مرض الشلل الرعاش. قد يساعد هذا في فهم أفضل للحالات المرضية والعصبية المختلفة مما قد يسمح بتصميم أساليب علاجية مخصصة لتحقيق نتائج علاجية أفضل للذكور والإناث