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# MONOAMİN OKSİDAZ AKTİVİTESİ VE NİTRİK OKSİT DÜZEYLERİ BEYNİN DEĞİŞİK BÖLGELERİNDE CİNSİYETLE İLİŞKİLİ DEĞİŞİM GÖSTERİR

MONOAMINE OXIDASE ACTIVITY AND NITRIC OXIDE LEVELS VARY BETWEEN DIFFERENT BRAIN REGIONS IN A GENDER SPECIFIC WAY

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**Anahtar Sözcükler**: monoamine oksidaz, nitrik oksit, cinsiyet, beyin, hipotalamus, hipokampus, MAO, NO

**Key Words**: monoamine oxidase, nitric oxide, sex, brain, hypothalamus, hippocampus, MAO, NO

## SUMMARY

*The discovery of morphological sex differences in bird and mammal brains led to extensive research on the possible effects of sex steroids on different areas of the CNS. The aim of our study was to investigate MAO activity and NO levels in different brain regions in order to determine whether there are any significant differences between sexes. MAO activity was lowest in amygdala and highest in the cortex in both male (n=8) and female (n=8) Sprague-Dawley rats, while it was found to be higher in the female hippocampus. Hypothalamus had the highest total nitrite-nitrate levels and was followed by the amygdala. While nitrite levels were higher in females, nitrate and total nitrite-nitrate levels were similar in all brain regions in both sexes. In the female cerebellum a (-) correlation between MAO activity and nitrite levels could be noted. Current study is the first report providing evidence on sex related regional differences in brain NO levels with respect to MAO activity. These gender specific variations might be taken into consideration for further research studies underlying neurodegenerative disorders and therapeutic strategies using MAO inhibitors as well as sex steroids.*

## ÖZET

Kuş ve memeli hayvanların beyinlerinde morfolojik cinsiyet farklılıklarının keşfi, beynin değişik bölgelerine seks-streroidlerinin olası etkileri konusunda ileri araştırmalara yol açmıştır. Bizim çalışmamızın da amacı, cinsiyetler arasında belirgin bir fark olup olmadığını ortaya koyabilmek için farklı beyin bölgelerindeki MAO aktivitelerini ve NO düzeylerini araştırmaktır. Sonuçlarda, hem erkek (n=8) hem dişi (n=8) Sprague-Dawley sıçanlarda, MAO aktivitesi amigdalada en düşük, kortekste en yüksek , hipotalamusta ise dişilerde daha yüksek bulundu. Total nitrit-nitrat seviyeleri en yüksek hipotalamusta saptanırken, bunu amigdala takip etti. Nitrit seviyeleri dişilerde yüksekken, nitrat ve total nitirt-nitrat seviyeleri açısından tüm beyin bölgelerinde erkeklerle dişiler arasında fark bulunamadı. Dişi serebellumunda MAO aktivitesi ile nitrit seviyeleri arasında negatif korelasyon izlendi. Bu çalışma MAO aktivitesi ile ilişkili olarak beyin NO seviyelerinde cinsiyete bağlı bölgesel farklılıkların kanıtlarını ortaya koyması açısından ilk bildiridir. Bu cinsiyete bağlı farklılıkların, nörodejeneratif hastalıklar ve MAO inhibitörleri ile seks-steroidleri ile tedavi yaklaşımları hakkında ileri araştırmalar yapılırken göz önüne alınması yararlı olabilir.

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## INTRODUCTION

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The discovery of morphological sex differences in bird and mammal brains led to extensive research on the possible effects of sex steroids on different areas of the central nervous system (CNS) (1, 2). Recent evidences obtained from basic and clinical studies point to alternating responses of the male and female brain to neurotoxic insults, ischemia and neurodegenerative diseases (3).

Positive impact of estrogen in neuroprotection was also documented (4). The beneficial effects of the hormone on neuroprotection were proposed to be by either direct mechanisms such as prevention of cell death and induction of axonal sprouting, augmenting regeneration of damaged neuronal connections, promoting synaptic transmission or indirect mechanisms such as lowering of cholesterol levels and improvement of immune function. The indirect effects are thought to be through actions on other organs which in turn affect the optimum function of the brain (2, 4). It has been documented that sex hormones affect the hippocampus which is important in learning and memory as well as in the effects of nitric oxide (NO) (5). NO is a gaseous biological messenger molecule also known as EDRF. Reports have shown that besides being a potent vasodilator, NO is a neurotransmitter / neuromodulator (6, 7) and that its effects might be associated with the actions of catecholamines. NO has been proposed to play a crucial role in the neurotoxic actions of glutamate and trans-synaptic regulation as well as in learning and memory processes (8). Monoamine oxidase (MAO [EC 1.4.3.4.; amine: oxygen oxidoreductase; deaminating, flavin containing]) a flavin-bound enzyme of the mitochondrial membrane due to its primary role in the metabolism of catecholamines, is a regulator of monoamine neurotransmitter levels in the brain as well as in the periphery (9). MAO exits in two forms, MAO A and MAO-B, coded by two different gene loci, with different patterns of tissue distribution (10). Its regulation is multifactorial and among hormonal factors, especially estrogens are speculated to have a crucial role as demonstrated by Holschneider et al. who found that high dose estrogen decreased MAO A activity in the hypothalamus by -28% and amygdale by -21% with no significant change in MAO-B and they suggested that estrogen exerts a tissue specific differential regulation of MAO A and B activity (11). Our previous work demonstrated the link between MAO and NO levels in the cardiac tissue of normal and aging male rats (12) as well as estrogen's and raloxifene's effects on MAO activity and NO levels in the brain of ovariectomized rats (13). The aim of our current work is to investigate MAO activity and NO levels in different brain regions in an attempt to assess whether there are any significant variations between male and female rats.

## METHODS

*Animals:*

Sprague-Dawley male (n=8) and female (n=8) rats weighing 225 ± 25 g were used in the study. Animals were housed in a temperature controlled room (20-25˚C), on a 12 h dark / 12 h light cycle and fed with standard laboratory chow and water ad libitum. Care and all treatment protocols complied with principles of Ege University Animal Research Ethics Committee.

*Chemicals:*

All chemicals were analytical grade. Kynuramine, 4-hydroxyquinoline, NADPH, FAD, sulphanilamide, N-1 naphtylenediamine, and bovine albumin standard were purchased from Sigma. Nitrate reductase from Aspergillus sp. was obtained from Boehringer-Mannheim. All the rest of the chemicals ($KH_2PO_4$, $K_2HPO_4$, 4-HQ, NaOH, $Na_2CO_3$, Na-K tartarate, $CuSO_4$, Folin– Chiocalteu reagents) were purchased from Merck & Darmstadt Co.

*Tissue preparation and analyses :*

Rats were decapitated, brains rapidly removed and dissected (cortex, hypothalamus, hippocampus, corpus striatum, cerebellum, amygdala) on ice. Tissues were stored at –70˚C till the day of analysis. On the day of analysis, tissue samples were weighed and homogenized on ice in 1/10 (w/v) of 0.5 M phosphate buffer (pH=7.4) by a Braun homogenizer. After centrifugation at 600 x g at 4°C for 10 minutes, supernatants were removed. MAO activity was determined fluorometrically with an Aminco-Bowman spectrofluorometer (Excitation: 318 nm, Emission: 385 nm) using kynuramine as substrate by Kraml's fluorometric method (14). Protein levels were determined by Lowry's method (15) and MAO activity results were expressed as nmol/mg protein/hour. Nitrite and nitrate levels were determined spectrophotometrically based on the Griess reaction (16) where nitrite reacts with sulfanilamide and N-(1-naphthyl) ethylendiamine to produce an azo dye detected at 543 nm, while nitrate is

first reduced to nitrite by nitrate reductase (EC 1.6.6.2.) via a reaction in which it is coupled to the oxidation of β-NADPH and detected at 340 nm. Sodium nitrite and nitrate solutions were used for standard measurements. Tissue nitrite and nitrate levels were expressed as nmol/mg protein.

*Statistics* :

Comparisons of means between brain regions were assessed by Friedman test-K related sample non-parametric test. Comparisons between sex groups were determined by Mann Whitney U test. Pearson correlation test was used for correlation analyses.

## RESULTS

*MAO activity :*

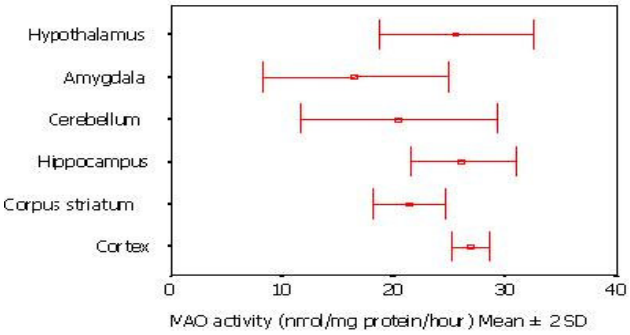


**Figure 1.** MAO activities in different brain regions of all rats in both sexes.

As presented in Figure 1. MAO activity was found to be significantly different in various brain regions, the lowest activity being in amygdale and highest in the cortex (Friedman test-K-related sample non-parametric test, P=0.019).

**Table I.** MAO activities of brain regions according to gender. Data are given as Mean ± SD (nmol/mg protein/hour)

| Brain regions | Female n=8 | Male n=8 |
|---|---|---|
| Hypothalamus | 22.20 ± 3.06 | 22.94 ± 17.07 |
| Amygdala | 13.39 ± 4.78 | 21.35 ± 20.08 |
| Cerebellum | 16.55 ± 4.01 | 28.08 ± 11.94 |
| Hippocampus | 28.57 ± 5.75 | 19.62 ± 10.36 |
| Corpus striatum | 23.09 ± 3.25 | 17.97 ± 14.45 |
| Cortex | 27.56 ± 2.28 | 29.13 ± 6.70 |

Region specific MAO activitiy results of male and female rat brains are shown in Table I. MAO activity was noted to be higher (p=0.059) in the female hippocampus as compared to males, while in male rats it was higher in the cerebellum as compared to females (p=0.081).

*Nitrite-nitrate levels :*

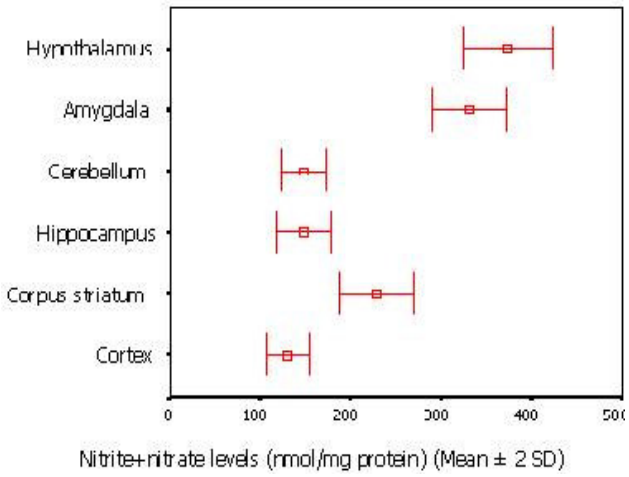


**Figure 2.** Total Nitrite+nitrate levels in different brain regions of all rats in both sexes.

Total nitrite-nitrate values are shown in Figure 2. As depicted, the hypothalamus, followed by the amygdala manifested the highest total nitrite-nitrate levels. Brain region specific nitrite and nitrate results of male and female rats are presented in Table II. As it is observed, nitrite levels of corpus striatum (p=0.065) and cortex (p=0.094) were higher in the females compared to males. While nitrite levels were higher in females compared to males, nitrate and total nitrite-nitrate levels were similar in all regions in both sexes.

**Table II.** Nitrite, nitrate and total nitrite+nitrate levels of brain regions according to gender. Data are given as Mean ± SD nmol/mg protein

| Brain regions | Female n=8 | | | Male n=8 | | |
|---|---|---|---|---|---|---|
| | Total | Nitrite | Nitrate | Total | Nitrite | Nitrate |
| Hypothalamus | 377 ± 75 | 5,0 ± 1,7 | 372 ± 73 | 376 ± 30 | 3,8 ± 1,7 | 372 ± 82 |
| Amygdala | 334 ± 57 | 3,7 ± 0,5 | 330 ± 57 | 331 ± 75 | 3,1 ± 0,7 | 328 ± 75 |
| Cerebellum | 135 ± 26 | 1,9 ± 0,5 | 133 ± 26 | 158 ± 46 | 1.5 ± 0,8 | 157 ± 46 |
| Hippocampus | 147 ± 18 | 1,8 ± 0,4 | 145 ± 18 | 138 ± 57 | 1.5 ± 0,6 | 136 ± 56 |
| Corpus striatum | 229 ± 61 | 2,9 ± 0,8 | 226 ± 61 | 212 ± 49 | 2.1 ± 0,8 | 210 ± 48 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Cortex | 119 ± 31 | 1,7 ± 0,4 | 117 ± 32 | 127 ± 39 | 1.3 ± 0,4 | 126 ± 38 |

*Correlation between MAO and NO :*

There was a significant correlation (P=0.02, r= -0.788) between MAO activity and nitrite levels in female cerebellum and a nonsignificant correlation (P=0.071, r=-0.666) in male hippocampus.

## DISCUSSION

Current study is the first report providing evidence on sex related regional differences in brain NO levels with respect to MAO activity. These regional variations and the possible link between NO and MAO may be beneficial while evaluating gender specific changes in conjunction with physiological functions such as learning and memory, as well as susceptibility to neurodegenerative diseases and their therapoetic approaches.

It has been well known that MAO show different patterns of tissue distribution (11). In accordance with their findings, our data has also revealed that MAO activity shows significant variation between various brain regions . In our study MAO activity was the lowest in amygdala while cortex MAO activity was the highest. When MAO activities of various brain regions were evaluated with regard to gender, a higher MAO activity was observed in the female hippocampus compared to males. This finding may be of special interest since the hippocampus plays an important role in memory and control of behaviour. It is also notable that the hippocampus, hypothalamus and amygdala are parts of the limbic system, associated with emotions. It seems likely that within the brain, information is transmitted from region to region and overall behavioural and emotional functions may depend on the cooperation of these regions (17).

Our results pointed out that the hypothalamus had the highest total nitrite-nitrate levels followed by the amygdala. These findings support the previous work of Barjavel and Bhargava who found the highest NOS activity of cerebrum in the hypothalamus, followed in decreasing order by the cortex, striatum, hippocampus and spinal cord (18). When our nitrite-nitrate levels were evaluated according to gender, female rats were found to have higher nitrite levels in all regions compared to males, while nitrate and total nitrite-nitrate levels were similar in all regions in both sexes.

A striking finding of our current work was the observation that in the hypothalamus with low MAO activity nitrite-nitrate values were highest. Other brain regions such as the hippocampus, cerebellum and cortex also manifested a similar inverse link. With regard to sex, there was a negative correlation (P=0.02, r=-0.788) between MAO activity and nitrite levels in the female cerebellum while

a nonsignificant correlation (P=0.071, r=-0.666) was noted also in the male hippocampus.

In the nervous system NO, different from classical neurotransmitters is a unique messenger acting in a nonsynaptic fashion. NO having the ability to penetrate to the cell membranes and affect neighboring neurons may participate in learning and memory processes via intra- and intercellular signalling. In addition to functioning as a neurotransmitter in the central and peripheric nervous system, NO plays a critical role in cerebral circulation. Endothelial dysfunction and impaired NO mediated vasodilation may contribute to the impairment of cognitive function in the elderly and also increase the susceptibility to neurodegenerative diseases. Impaired NO production has been reported in the CNS of patients with Alzheimer's disease (AD) and Parkinson's disease (3,19). Reduced synthesis of NO in neurodegenerative diseases implies that administration of agents leading to enhanced production of NO might be of therapeutic value in such pathologies. Recently L-deprenyl, a MAO inhibitor, was shown to induce rapid increases in NO production in brain tissue and cerebral blood vessels. Since NO modulates activities including cerebral blood flow and memory, and reduced NO production has been observed in AD brain, stimulation of NO production by l-deprenyl, a selective MAO-B inhibitor could contribute to the improvement of cognitive function in AD (10). MAO inhibition could prevent the generation of hydrogen peroxide and oxygen radicals thus ameliorating the NO/oxidative stress imbalance and cerebral circulation.

The sex related regional differences and striking negative link between MAO activity and NO levels in different brain regions observed in our study may be encouraging for further research studies underlying neurodegenerative disorders and also promising for possible therapeutic strategies using MAO inhibitors as well as sex steroids, particularly estrogen.

## CONCLUSION

The link between MAO activity and NO levels detected in different brain regions with respect to gender may be due to the specific physiological roles of these variables in alternating areas as well as the presence of a common pathway regulating both variables.

Our data demonstrating gender specific variations in MAO activity and NO levels support the modulatory role of sex steroids on MAO activity and NO. We propose that gender specific changes have to be considered in further research studies underlying neurodegenerative disorders and therapeutic strategies using MAO inhibitors as well as sex steroids.

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