# Brain Injury and Inflammation Genes Common to a Number of Neurological Diseases and the Genes Involved in the Genesis of GABAnergic neurons Are Altered in Monoamine Oxidase B Knockout Mice

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**Abbreviations:** MAO, monoamine oxidase; DEG, differentially expressed gene; IPA, Ingenuity Pathway Analysis; PEA, phenylethylamine; OCD, obsessive compulsive disorder; ALS, amyotrophic lateral sclerosis; ADHD, attention deficit hyperactive disorder; GABA, gamma-aminobutyric acid

# Abstract

Monoamine oxidase B (MAO B) oxidizes trace amine phenylethylamine (PEA), and neurotransmitters serotonin and dopamine in the brain. We reported previously that PEA levels increased significantly in all brain regions, but serotonin and dopamine levels were unchanged in MAO B knockout (KO) mice. PEA and dopamine are both synthesized from phenylalanine by aromatic L-amino acid decarboxylase in dopaminergic neurons in the striatum. In the absence of MAO B, a high concentration of PEA in the striatum may cause dopaminergic neuronal death. In this study, we isolated the RNA from brain tissue of MAO B KO mice (2-month old) and age-matched wild type (WT) male mice and analyzed the altered genes by Affymetrix microarray. Differentially expressed genes (DEGs) in MAO B KO compared to WT mice were analyzed by Partek Genomics Suite, followed by Ingenuity Pathway Analysis (IPA) to assess their functional relationships. DEGs in MAO B KO mice are involved in brain inflammation and the genesis of GABAnergic neurons. The significant DEGs include five brain injury or inflammation genes (upregulated: *Ido1, TSPO, AVP, Tdo2),* five gamma-aminobutyric acid (GABA) receptors (down-regulated: *GABRA2, GABRA3, GABRB1, GABRB3, GABRG3),* six transcription factors related to adult neurogenesis (upregulated: *Wnt7b, Hes5;* down-regulated: *Pax6, Tcf4, Dtna).* Altered brain injury and inflammation genes in MAO B knockout mice are involved in various neurological disorders: attention deficit hyperactive disorder (ADHD, 11/54 genes), panic disorder (14/38 genes), obsessive compulsive disorder (OCD: 18/59 genes), autism (25/74 genes), amyotrophic lateral sclerosis (ALS: 32/83 genes), Parkinson's diseases (50/170 genes), Alzheimer's disease (65/237genes), bipolar affective disorder (72/250 genes), where X and Y of (X/Y) denote the user input genes overlapping with a disorder and a total number of genes

present in Qiagen knowledge database overlapping with the disorder, respectively. A number of altered genes were commonly involved in these psychiatric and neurological disorders, indicating that there are overlapping molecular pathways in these disorders.

**Keywords:** monoamine oxidase B, phenylethylamine, inflammation, GABAnergic neuron

## 1. Introduction

Monoamine oxidase (MAO) A and B are mitochondria-bound isoenzymes that catalyze the deamination of dietary amines and monoamine neurotransmitters (Bach et al., 1988, Shih, 2018, Shih et al., 2018). MAO A has a higher affinity for serotonin [5-hydroxytryptamine (5-HT)] and norepinephrine (NE) whereas phenylethylamine (PEA) and benzylamine are the preferred substrates of MAO B. Both isoenzymes degrade dopamine (DA), tyramine, and tryptamine (Shih et al, 1999).

In MAO B knockout (KO) mice, PEA levels increased significantly in all brain regions but serotonin and dopamine levels were unchanged (Grimsby et al., 1997), which confirms the role of MAO B in the catabolism of PEA. PEA and dopamine are both synthesized from phenylalanine by aromatic L-amino acid decarboxylase in dopaminergic neurons in the striatum. In the absence of MAO B, the highest increase in PEA was found in the striatum (Bortolato *et al.,* 2009), which affects adult neural stem cell niches in the subventricular zone of the lateral ventricle and has been shown associated with dopaminergic neuron death.

The etiological basis of a number of neurodegenerative disorders including Parkinson's disease and Alzheimer's disease remains largely unknown. With aging, an increase in MAO B level occurs (Fowler et al., 1980; Kumar et al. 2004), indicative of its potential role in the cognitive decline associated with aging-related neurological diseases (Santin et al., 2021; Tripathi et al., 2018). Consistently, an increased level of MAO B in the brain has been observed in both Alzheimer's disease and Parkinson's disease (Mallajosyula et al., 2009; Saura et al., 1994). As MAO B is predominantly expressed in glial cells (Levitt et al., 1982; Westlund et al, 1985), the increased oxidation of PEA by MAO B, resulting in the generation of reactive oxygen

species (ROS), may contribute to the loss of dopaminergic neurons in the substantia nigra, which is associated with Parkinson's disease.

Enhanced MAO B activity has also been implicated in cardiac dysfunction (Kaludercic et al., 2014; Maggiorani et al., 2017). Polymorphisms in MAO B gene have been associated with negative emotionality that causes depression (Dlugos et al., 2009). The MAO B inhibitor deprenyl (selegiline) has shown efficacy in ameliorating depressive symptoms (Mendelewicz, et al., 1983; Ziegler et al., 2018; Karabelyov et al., 2021), improving ADHD associated with Tourette's syndrome (Jankovic et al., 1993; Feigin et al., 1996), and slowing the Alzheimer's disease progression (Sano et al., 1997).

MAO B KO mice display increased reactivity to stress (Grimsby et al., 1997) and lower levels of anxiety-like behaviors (Bortolato et al., 2009). MAO B KO mice are also resistant to the Parkinsongenic neurotoxin, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), indicative of the role of MAO B in this disorder (Grimsby et al., 1997). MPTP is converted by MAO B to the toxic metabolite $MPP^+$ (1-methyl-4-phenylpyridine), which selectively destroys nigrostriatal neurons. (Kopin et al 1988).

MAO B KO mice exhibit physiological and behavioral alterations that may be induced by the increased PEA level, which includes alteration in the distribution of cerebral blood flow (Scremin et al., 1999) and attenuation of behavioral responses to amphetamine (Yin et al., 2006). PEA and other trace amines have been implicated in schizophrenia, mania, and attention deficit hyperactivity disorder (ADHD) (Wolinksky et al., 2007). PEA was shown to function as the main activator of trace-amine associated receptor 1 (TAAR1) to modulate catecholamine signaling (Xie et al., 2008). These results suggest that MAO B KO mice may serve as a model to study the long-term effects of elevated PEA levels on brain function and behavior. The

present study was undertaken to identify differentially expressed genes in 2-month old MAO B KO mice to gain insight into the mechanism through which increased PEA causes the above phenotypes.

## 2. Results

### 2.1. Gene expression changes in MAO B knockout mice

The gene expression profiles of age-matched MAO B KO and WT mice were determined by microarray analysis. RNA was isolated from the brain tissues of 2-month old mice and converted to cDNA to generate probes (targets) (Tabakoff *et al.,* 2008). Targets corresponding to individual mutant or WT mice were hybridized with Affymetrix GeneChip arrays. Partek Genomics Suite was then used to identify genes with altered expression in the MAO B mutant. To avoid excluding small yet significant changes in gene expression, DEGs were defined as those with a false discovery rate (FDR) < 0.05 and a fold-change (MAO B KO versus WT mice) >1.5 or -1.5 for the analysis. The above analysis identified many genes with statistically significant differential expression in MAO B KO compared to WT mice.

### 2.2 Differentially expressed genes in MAO B KO mice are involved in brain inflammation and the genesis of GABAnergic neuron

Over 6 fold increase in five genes which are involved in brain injury or inflammation were found in MAO B KO mice compared to WT littermates: indoleamine-2,3-dioxygenase (Ido1: +14 fold), 18 kd translocator protein (TSPO, +9 fold), arginine vasopressin (AVP, +7 fold), tryptophan 2,3-dioxygenase (Tdo2, +6 fold) (Table 1). The increase in these marker genes was associated with

the decrease of five gamma-aminobutyric acid (GABA) receptors (GABRA2, -4 fold; GABRA3, -2 fold; GABRB1, -3 fold; GABRB3, -13 fold; GABRG3, -16 fold) (Table 2) and the alteration of six transcription factors, which are related to adult neurogenesis (Pax6, -4 fold; Tcf4, -4 fold; Wnt7b, +5 fold; Hes5, +9 fold; Dtna, -8 fold) (Table 3).

### 2.3 Differentially expressed genes in MAO B KO mice are linked to various neurological disorders

Functional annotation database of Ingenuity Pathways Analysis was used to identify genes linked to various neurological diseases. DEGs linked to brain injury or inflammation and the following disorders were identified using the IPA database: attention deficit hyperactive disorder (ADHD, 11/54 genes), panic disorder (14/38 genes), obsessive compulsive disorder (OCD: 18/59 genes), autism (25/74 genes), amyotrophic lateral sclerosis (ALS: 32/83 genes), Parkinson's diseases (50/170 genes), Alzheimer's disease (65/237genes), bipolar affective disorder (72/250 genes) where X and Y of (X/Y) denote the user input genes overlapping with a disorder and a total number of genes present in Qiagen knowledge database overlapping with the disorder, respectively. Figure 1 displays the DEGs identified in 2-month old MAO B KO mice that are relevant to the disorders.

Attention deficit hyperactive disorder-linked genes that were differentially expressed include ADRA2A (alpha-2A adrenergic receptor), ADRA2C (alpha-2C adrenergic receptor), CHRM1 (muscarinic acetylcholine receptor $M_1$), GABRA2 (GABA receptor subunit alpha-2), GABRA3 (GABA receptor subunit alpha-3), GABRB1 (GABA receptor subunit beta-1), GABRB3 (GABA receptor subunit beta-3), GABRG1 (GABA receptor subunit gamma-1),

GABRG3 (GABA receptor subunit gamma-3), HTR2C (5-HT receptor 2C), and TSPO (translocator protein).

Panic disorder-linked genes that were altered include ADRA2A, ADRA2C, GABRA2, GABRA3, GABRB1, GABRB3, GABRG1, GABRG3, the G protein-regulated inducer of neurite outgrowth (GRIN) family members GRIN2B [glutamate (NMDA) receptor subunit 2B], GRIN2C [glutamate (NMDA) receptor subunit 2C], GRIN2D [glutamate (NMDA) receptor subunit 2D], GRIN3A [glutamate (NMDA) receptor subunit 3A], MAOA (monoamine oxidase A), and TSPO.

Differentially expressed genes involved in obsessive compulsive disorder include ADRA2A, ADRA2C, CA3 (carbonic anhydrase 3), GRIN2B, GRIN2C, GRIN2D, GRIN3A, HTR2C, HTT (huntingtin), MAOA, SCN1A (sodium channel, voltage-gated, type I, alpha subunit), SCN1B (sodium channel subunit beta-1), SCN2A (sodium channel, voltage-gated, type II, alpha subunit), SCN3B (sodium channel subunit beta-3), SCN8A (sodium channel, voltage-gated, type VIII, alpha subunit), SCN9A (sodium ion channel $Na_v1.7$), SLC1A1 (excitatory amino-acid transporter 3), and SLC1A3 [solute carrier family 1 (glial high-affinity glutamate transporter), member 3].

Autism-liked genes with altered expression include ACHE (acetylcholinesterase), ADRA2A, ADRA2C, CHRM1, GABRA2, GABRA3, GABRB1, GABRB3, GABRG1, GABRG3, GAD2 (glutamate decarboxylase 2), HTR2C, SCN1A, SCN1B, SCN2A, SCN3B, SCN8A, SCN9A, SLC1A1, SLC1A3, and TSPO.

Differentially expressed genes associated with amyotrophic lateral sclerosis include BCL2 (B-cell lymphoma 2), GAD2, GRIN2B, GRIN2C, GRIN2D, GRIN3A, HMGCR (HMG CoA reductase), IGF-1 (insulin-like growth factor 1), SCN1A, SCN1B, SCN2A, SCN3B,

SCN8A, SCN9A, SLC1A1, SLC1A3, and TRPM7 (transient receptor potential cation channel, subfamily M, member 7).

Parkinson's disease-linked genes that were differentially expressed include ACHE, ADRA2A, ADRA2C, BCL2, CA3, CACNA1C (calcium channel, voltage-dependent, L type, alpha 1C subunit), CACNA1D (calcium channel, voltage-dependent, L type, alpha 1D subunit), CHRM1, GABRA2, GABRA3, GABRB1, GABRB3, GABRG1, GABRG3, GRIA1 (glutamate receptor 1), GRIA3 (glutamate receptor 3), GRIA4 (glutamate receptor 4), GRIN2B, GRIN2C, GRIN2D, GRIN3A, HNRPDL (heterogeneous nuclear ribonucleoprotein D-like), HTR2C, MAOA, MAOB, MAPT (Tau protein), and TRPM7.

Alzheimer's disease-linked genes that were altered include ACHE, ADRA2A, ADRA2C, CACNA1C, CACNA1D, CHRM1, GAD2, GRIA1, GRIA3, GRIA4, GRIN2B, GRIN2C, GRIN2D, GRIN3A, HMGCR, HNRPDL, HTR2C, HTT, IGF1, MAOA, MAOB, MAPT, NOS3 (nitric oxide synthase 3), and NR3C1 (nuclear receptor subfamily 3, group C, member 1; glucocorticoid receptor).

Differentially expressed genes related to the bipolar disorder include ADRA2A, ADRA2C, CA3, CHRM1, GABRA2, GABRA3, GABRB1, GABRB3, GABRG1, GABRG3, GAD2, GRIN2B, GRIN2C, GRIN2D, GRIN3A, HTR2C, MAOA, NDUFV2 [NADH dehydrogenase (ubiquinone) flavoprotein 2], NOS3, NR3C1, SCN1A, SCN1B, SCN2A, SCN3B, SCN8A, SCN9A, SLC1A1, SLC1A3, and TSPO.

The results from our microarray analysis clearly indicate that many of the DEGs identified in MAO B KO mice are involved in the pathobiology of multiple neurological disorders. To gain further insight into the underlying mechanism, IPA was used to cluster functionally related genes corresponding to specific canonical pathways. Figure 2 illustrates

connectivity between genes differentially expressed in 2-month old MAO B KO mice and their relation to several functional categories.

## 3. Discussion

This is the first study demonstrating the role of MAO B in brain inflammation, and adult stem cell neurogenesis circuitry. It further describes that brain Injury and inflammation genes altered in MAO B KO mice are involved in the common molecular pathways in a number of psychiatric and neurological disorders (Ostadkarampour et al., 2021). These results provide novel function of PEA in adult neurogenesis and GABA receptor expression, which will provide insights into neurodegenerative diseases, such as Parkinson's disease (Naoi et al., 2020; Romero et al., 2020; Robakis et al., 2015). In addition, the downregulation of GABA receptors in MAO B KO mice is consistent with lower anxiety-like responses and shorter latency to engage in risk-taking behavior in MAO B KO mice compared with WT littermates (Bortolato *et al.*, 2009).

Idol1 was found to have a 14 fold increase in MAO B KO mice compared to their WT littermates. The Idol1 gene encodes an immunomodulatory enzyme produced by macrophages and other immune cells. It catalyzes the conversion of L-tryptophan into N-formylkynurenine. The depletion of L-tryptophan leads to the suppression of growth. TSPO encodes another immunomodulatory protein that is associated with the inflammatory response after brain injury and several neurological diseases. Its 9 fold increase in MAO B KO mice indicates that there is an inflammatory process taking place in the presence of high levels of PEA. AVP encodes a hormone, whose major function is to regulate water retention. However, studies show that it is also implicated in pair-bonding and aggression. Some controversial studies suggest that AVP plays a role I memory formation and delayed reflexes. Tdo2, like Ido1, encodes an enzyme that

catalyzes the conversion of L-tryptophan. Its 6 fold increase in MAO B KO mice suggests that there is further depletion of L-tryptophan, which may be causing some suppression of cellular growth.

The GABA(A) receptor is a ligand-gated ion channel. Its major function is to provide inhibitory neurotransmission in the brain. It has a pentameric structure, which usually consists of 2$\alpha$, 2$\beta$, and 1$\gamma$ subunits. Various drugs use this receptor as a target to reduce anxiety and to induce sedative effects. Our microarray data shows that the GABA(A) receptor subunits expression is decreased after exposure to high levels of PEA. Possibly, the increase in inflammatory genes may be associated with decreased expression of GABA(A) receptor subunits. This also suggests that MAO B KO mice should have more excitatory neurotransmission than their WT littermates.

Dystrobrevin (Dtna) gene encodes a protein that is thought to be associated with the formation and stability of synapses. Specifically, it decreases the maturation and stability of postsynaptic density in the neuromuscular junction. Thus, in MAO B KO mice, we expect to see more mature and stable postsynaptic density than in WT littermates. Paired box 6 (Pax6) encodes a very important transcription factor that is critical for neurogenesis. Its 4 fold decrease in MAO B KO mice suggests that exposure to high levels of PEA could lead to deficits in neuronal development. Wingless-related MMTV integration site 7B (Wnt7b) encodes a protein involved in increasing the maturation and proliferation of neuronal progenitor cells. The 5 fold increase indicates that MAOB KO may have a role for neuronal precursor cells. Hairy and enhancer split 5 (Drosophila) or Hes5 encodes a protein that regulates cell differentiation. It decreases the differentiation of neurons. So exposure to high levels would possibly lead to greater differentiation of neurons. Thus, our study shows that MAO B KO mice have altered

expression levels of genes that affect neurogenesis, which can be attributed to long-term exposure to high levels of PEA.

Figure 3 illustrates connectivity between genes differentially expressed in 2-month old MAO B KO mice with relevance to inflammation. Interestingly, there were 44 altered genes between MAO B KO mice and WT mice out of 134 anxiety and anxiety-like behavior disorder. GABA receptors, TSPO and AVP are shown in Fig. 3. GABA transporter (SLC6A13) increased 12 fold (p-value 3.321E-7) but did not find a direct connection through IPA.

Attention deficit hyperactivity disorder is a neurological disorder characterized by impulsive behavior, short attention span, and inability to focus. GABA neurotransmission in the brain is a major form of inhibitory control and plays a role in regulating behavior. Our analysis shows that MAO B KO mice have altered levels of GABA(A) subunits, which are also implicated in ADHD. GABA(A) receptors are ligand-gated chloride channels that cause hyperpolarization when activated. The GABA(A) receptors are usually pentameric with two $\alpha$, two $\beta$, and a $\gamma$ subunit. In MAO B KO mice, there is a decrease in the expression of $\alpha 2$, $\alpha 3$, $\beta 1$, $\beta 3$, and $\gamma 3$ subunits, and an increase in $\gamma 1$ subunit. New pharmacological approaches are now focusing on modulating and activating these subunits to treat ADHD. Eszopiclone (Lunesta®) and zolpidem (Amnien®) are Food and Drug Administration (FDA) approved agents for insomnia that are currently in Phase III trials for ADHD treatment. These agents act as agonists and modulators of the GABA(A) subunits to inhibit excitatory neurotransmission. Since the MAO B KO mice have mostly decreased expression of the GABA(A) subunits, the MAO B KO mice may have less inhibition in neurotransmission than their WT littermates.

Panic disorder is a neurological disorder characterized by anxiety and severe panic attacks. Currently, benzodiazepines such as clonazepam (Klonopin®) and alprazolam (Zanax®)

are approved by FDA for treating panic disorder. They work as agonists and modulators of the GABA(A) receptor. However, possible new pharmacological therapy includes agonists of the glutamate ionotropic NMDA receptor (GRIN). Cycloserine (Seromcin®), a GRIN agonist, is an antimicrobial agent that is currently in Phase II trials for panic disorder and in Phase II for obsessive compulsive disorder. Our microarray analysis revealed that there is a decrease in GRIN2B and 3A and an increase in GRIN2C and 2D expression in MAO B KO mice. During normal development, the high expression of GRIN2B is replaced by GRIN2A. In the MAO B KO mice, there is no significant change in GRIN2A; however, GRIN2B is lower than in WT littermates. This decrease may play a role in hippocampal plasticity and thus play a role in memory formation.

Obsessive compulsive disorder is a neurological disorder characterized by compulsive repetitive behavior and anxiety. Current treatment includes select serotonin reuptake inhibitors (SSRIs) and benzodiazepines. Unlike cycloserine, which is a GRIN agonist, memantine (Namenda®) is a GRIN antagonist approved for Alzheimer's disease and in Phase III trials for OCD and ALS. However, riluzone (Rilutek®), an agent approved for ALS, is a sodium channel (SCN) and solute carrier (SLC) inhibitor that is currently in Phase II trials for the treatment of OCD, bipolar disorder, and autism. In our study, the MAO B KO mice were found to have decreased expression of voltage-gated sodium channels (SCN1A, SCN2A, SCN3B, SCN8A) and an increase in SCN1B and SCN9A expression. These channels are responsible for the rising phase of the action potential in neurons. Unlike the $\beta$ subunit, $\alpha$ subunit can function on its own. In addition, the MAO B KO mice have decreased expression in solute carrier family 1 (high-affinity glutamate transporter). A decrease in this transporter may indicate that MAO B KO mice are not getting as much glutamate into their cells as their WT littermates.

This study demonstrates that there are overlapping molecular pathways in brain inflammation and these neurological disorders. For instance, five GABA(A) receptor subunits (GABRA2, GABRA3, GABRB1, GABRB3, GABRG3) were decreased in MAO B KO mice, which are involved in ADHD and panic disorder. Four ionotropic glutamate receptors (GRIN2B, GRIN3A decreased; GRIN2C, GRIN2D were increased) were altered in MAO B KO mice, which are related to panic disorder and OCD. A decrease in voltage-gated sodium channels (SCN1A, SCN2A, SCN3B, SCN8A) and an increase in SCN1B and SCN9A were observed in MAO B KO mice. These genes are common in OCD, ALS, autism, bipolar affective disorder, Parkinson's disease, and Alzheimer's disease. Since MAO B is located in glia and serotonergic neuron only, the altered expression of a large number of genes found in MAO B KO mice would most likely be located in glia and serotonergic neurons.

Lastly, we note that the RT-PCR regional brain analysis did not reveal the same level of change as the whole brain microarray analysis (data not shown). In addition, some of the tested genes produced multiple PCR products, suggesting that there are splicing variations of these genes. Future studies should investigate the cause and impact of such variations. Future tests should also investigate the other genes that interact with the genes in this study to get a complete picture of regional gene expression.

# 4. Experimental Procedures

## 4.1 Microarray analysis

RNA was isolated from the brain tissue of MAO B KO (2-month old) and age-matched WT mice and converted to cDNA to generate probes for hybridization (Saba et al., 2006; Tabakoff et al., 2008). Gene expression levels were determined using Affymetrix GeneChip® arrays (Santa Clara, CA). Partek Genomics Suite (PGS) (St. Louis, MO) was used for statistical analysis of microarray data to identify differentially expressed genes between MAO B KO and WT mice (n=5). Differentially expressed genes (DEGs) are defined as MAO B KO vs WT [false discovery rate (FDR) < 0.05, fold-change (FC) >1.5]. All procedures performed were approved by the Institutional Animal Care and Use Committee of the University of Southern California.

## 4.2 Animals

MAO B KO mouse was previously generated using a gene-targeting vector. MAO B was inactivated through the insertion of a transcriptionally active neomycin resistance gene into exon 6. It introduced a stop codon that resulted in a truncated, inactive MAO B enzyme. (Grimsby *et al*., 1997). The mice were maintained on a 12 h day/light cycle with access to food and water following a protocol approved by the University of Southern California Institutional Animal Care and Use Committee (IACUC).

## 4.3 Computational analysis of protein functions

The DEG data were further analyzed with Ingenuity Pathway Analysis (IPA) software (Redwood City, CA), which integrates the microarray data with known disease pathways to create a map of gene expression related to neurological diseases.

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# Figures & Legends

| GENES | ADHD | Panic Disorder | OCD | Autism | ALS | Parkinson's Disease | Alzheimer's Disease | Bipolar Disorder |
|---|---|---|---|---|---|---|---|---|
| ACHE | | | | ACHE | | ACHE | ACHE | |
| ADRA2A | ADRA2A | ADRA2A | ADRA2A | ADRA2A | | ADRA2A | ADRA2A | ADRA2A |
| ADRA2C | ADRA2C | ADRA2C | ADRA2C | ADRA2C | | ADRA2C | ADRA2C | ADRA2C |
| BCL2 | | | | | BCL2 | BCL2 | | |
| CA3 | | | CA3 | | | CA3 | | CA3 |
| CACNA1C | | | | | | CACNA1C | CACNA1C | |
| CACNA1D | | | | | | CACNA1D | CACNA1D | |
| CHRM1 | CHRM1 | | | CHRM1 | | CHRM1 | CHRM1 | CHRM1 |
| GABRA2 | GABRA2 | GABRA2 | | GABRA2 | | GABRA2 | | GABRA2 |
| GABRA3 | GABRA3 | GABRA3 | | GABRA3 | | GABRA3 | | GABRA3 |
| GABRB1 | GABRB1 | GABRB1 | | GABRB1 | | GABRB1 | | GABRB1 |
| GABRB3 | GABRB3 | GABRB3 | | GABRB3 | | GABRB3 | | GABRB3 |
| GABRG1 | GABRG1 | GABRG1 | | GABRG1 | | GABRG1 | | GABRG1 |
| GABRG3 | GABRG3 | GABRG3 | | GABRG3 | | GABRG3 | | GABRG3 |
| GAD2 | | | | GAD2 | GAD2 | | GAD2 | GAD2 |
| GRIA1 | | | | | | GRIA1 | GRIA1 | |
| GRIA3 | | | | | | GRIA3 | GRIA3 | |
| GRIA4 | | | | | | GRIA4 | GRIA4 | |
| GRIN2B | | GRIN2B | GRIN2B | | GRIN2B | GRIN2B | GRIN2B | GRIN2B |
| GRIN2C | | GRIN2C | GRIN2C | | GRIN2C | GRIN2C | GRIN2C | GRIN2C |
| GRIN2D | | GRIN2D | GRIN2D | | GRIN2D | GRIN2D | GRIN2D | GRIN2D |
| GRIN3A | | GRIN3A | GRIN3A | | GRIN3A | GRIN3A | GRIN3A | GRIN3A |
| HMGCR | | | | | HMGCR | | HMGCR | |
| HNRPDL | | | | | | HNRPDL | HNRPDL | |
| HTR2C | HTR2C | | HTR2C | HTR2C | | HTR2C | HTR2C | HTR2C |
| HTT | | | HTT | | | | HTT | |
| IGF1 | | | | | IGF1 | | IGF1 | |
| MAOA | | MAOA | MAOA | | | MAOA | MAOA | MAOA |
| MAOB | | | | | | MAOB | MAOB | |
| MAPT | | | | | | MAPT | MAPT | |
| NDUFV2 | | | | | | | | NDUFV2 |
| NOS3 | | | | | | | NOS3 | NOS3 |
| NR3C1 | | | | | | | NR3C1 | NR3C1 |
| SCN1A | | | SCN1A | SCN1A | SCN1A | | | SCN1A |
| SCN1B | | | SCN1B | SCN1B | SCN1B | | | SCN1B |
| SCN2A | | | SCN2A | SCN2A | SCN2A | | | SCN2A |
| SCN3B | | | SCN3B | SCN3B | SCN3B | | | SCN3B |
| SCN8A | | | SCN8A | SCN8A | SCN8A | | | SCN8A |
| SCN9A | | | SCN9A | SCN9A | SCN9A | | | SCN9A |
| SLC1A1 | | | SLC1A1 | SLC1A1 | SLC1A1 | | | SLC1A1 |
| SLC1A3 | | | SLC1A3 | SLC1A3 | SLC1A3 | | | SLC1A3 |
| TSPO | TSPO | TSPO | | TSPO | | | | TSPO |
| TRPM7 | | | | | TRPM7 | TRPM7 | | |

**Figure 1**

**Altered expression of genes linked to neurological disorders in 2-month old MAO B KO mice.** Ingenuity Pathways Analysis (IPA) annotation database was used to identify differentially expressed genes (DEGs) linked to various neurological disorders [false discovery rate (FDR) < 0.05; fold-change (FC) >2].

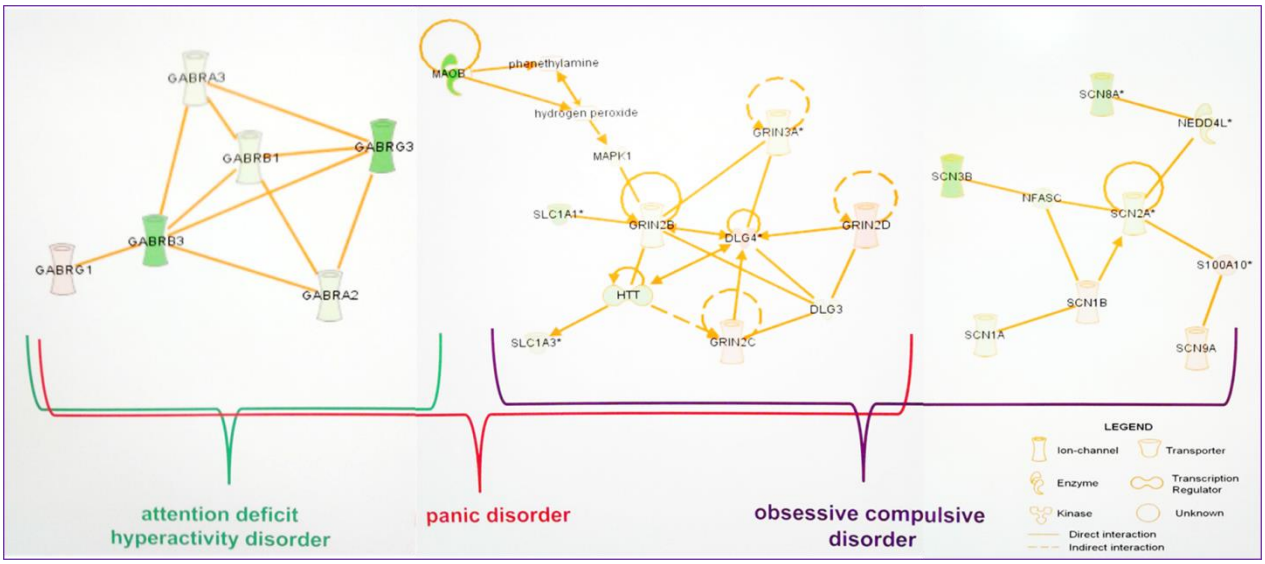


**Figure 2**

**Pathways generated by Ingenuity Pathway Analysis for altered genes in 2-month old MAO B KO mice.** The green color indicates decreased expression and red indicates increased expression. Color intensity corresponds to the fold change. The lines connecting the GABA receptor (GABR) subunits indicate protein-protein interactions.

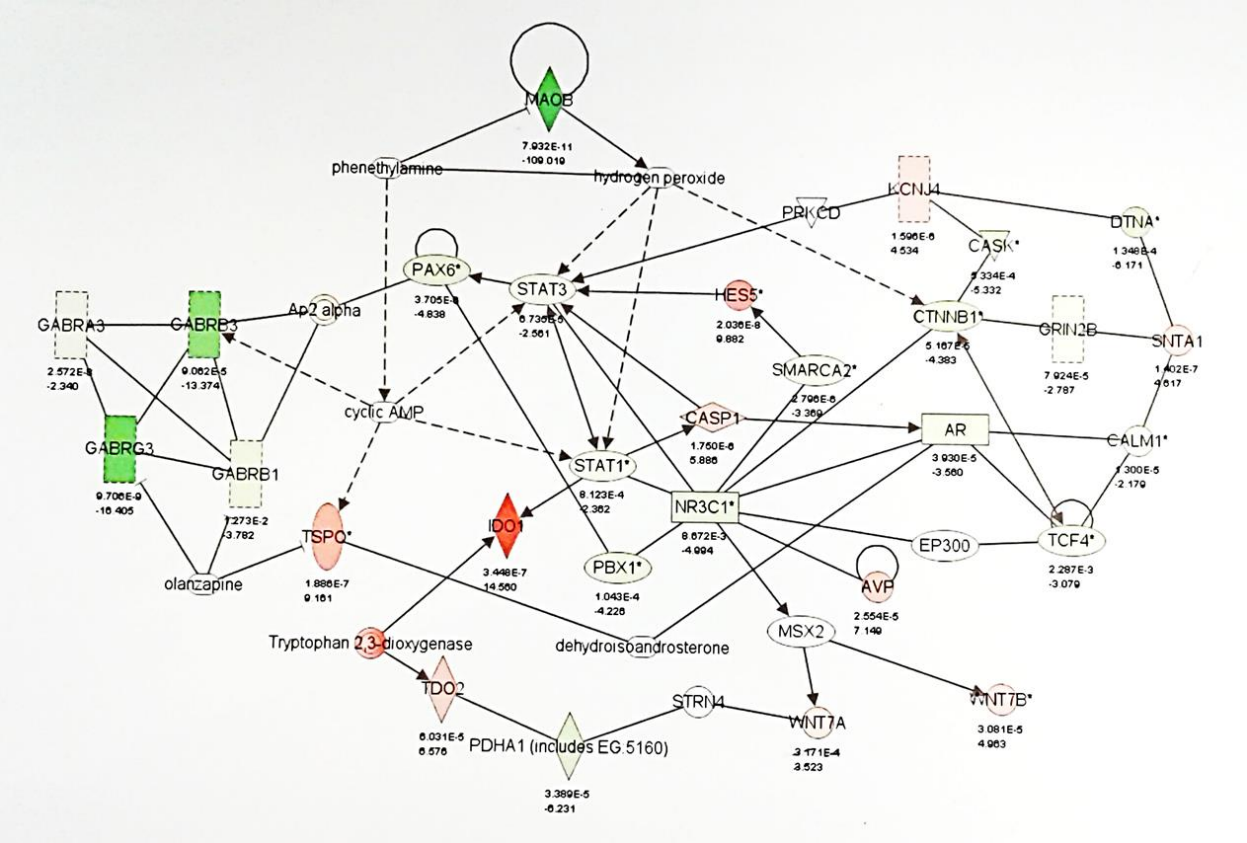


**Figure 3**

Signal transduction pathways of MAO B KO mice including inflammation genes, GABA receptors, and transcription factors related to neuron stem cells are listed in Table 1-3. The other genes altered between MAO B KO mice and WT mice were also included. Small molecules and other genes without change were added to complete the pathway. The broken line: indirect relation; filled line: direct protein-protein interaction.

# Tables

| GENE | FOLD CHANGE | P-VALUE |
|---|---|---|
| **Ido1** (indoleamine-2,3-dioxygenase) | +14 | 3.45E-07 |
| **TSPO** (18kd translocator protein) | +9 | 1.89E-07 |
| **AVP** (arginine-Vasopressin) | +7 | 2.55E-05 |
| **Tdo2** (tryptophan 2,3-dioxygenase) | +6 | 6.03E-05 |

**Table 1**

**Increased expression of genes involved in inflammation and brain injury in 2 month-old MAO B KO mice.**

Partek Genomics Suite was used for statistical analysis of microarray data to identify differentially expressed genes between MAO B KO and WT mice. Differentially expressed genes (DEGs) are defined as MAO B KO vs WT [false discovery rate (FDR) < 0.05, fold-change (FC) >1.5].

| GABA RECEPTORS | FOLD CHANGE | P-VALUE |
|---|---|---|
| **GABRA2** [gamma-aminobutyric acid (GABA) A receptor, alpha2] | -4 | 2.58E-03 |
| **GABRA3** [gamma-aminobutyric acid (GABA) A receptor, alpha3] | -2 | 2.57E-08 |
| **GABRB1** [gamma-aminobutyric acid (GABA) A receptor, beta 1] | -3 | 1.27E-02 |
| **GABRB3** [gamma-aminobutyric acid (GABA) A receptor, beta 3] | -13 | 9.06E-05 |
| **GABRG3** [gamma-aminobutyric acid (GABA) A receptor, gamma 3] | -16 | 9.71E-09 |

**Table 2**

**Decreased expression of GABA receptor subunits in 2 month-old MAOB KO mice.**

Partek Genomics Suite was used for statistical analysis of microarray data to identify differentially expressed genes between MAO B KO and WT mice. Differentially expressed genes (DEGs) are defined as MAO B KO vs WT [false discovery rate (FDR) < 0.05, fold-change (FC) >1.5].

| TRANSCRIPTION FACTORS<br>Related to neurogenesis | FOLD CHANGE | P-VALUE |
|---|---|---|
| **Dtna**<br>(dystrobrevin, alpha) | -8 | 1.35E-04 |
| **Pax6**<br>(paired box 6) | -4 | 3.71E-06 |
| **Tcf4**<br>(transcription factor 4) | -4 | 4.73E-06 |
| **wnt7b**<br>(wingless-related MMTV integration site 7B) | +5 | 2.01E-06 |
| **Hes5**<br>[hairy and enhancer of split 5 (Drosophila)] | +9 | 3.18E-08 |

**Table 3**

**Altered expression of transcription factors related to neurogenesis in 2 month-old MAOB KO mice.**

Partek Genomics Suite was used for statistical analysis of microarray data to identify differentially expressed genes between MAO B KO and WT mice. Differentially expressed genes (DEGs) are defined as MAO B KO vs WT [false discovery rate (FDR) < 0.05, fold-change (FC) >1.5].