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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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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. A high concentration of PEA in the striatum may cause dopaminergic neuronal death in the absence of MAO B. 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 four brain injury or inflammation

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genes (upregulated: *Ido1, TSPO, AVP, Tdo2),* five gamma-aminobutyric acid (GABA) receptors (down-regulated: *GABRA2, GABRA3, GABRB1, GABRB3, GABRG3),* five 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, panic disorder, obsessive compulsive disorder, autism, amyotrophic lateral sclerosis, Parkinson's diseases, Alzheimer's disease, bipolar affective disorder. Many were commonly involved in these disorders, indicating that there are overlapping molecular pathways.

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. The MAO A and MAO B isoenzymes degrade dopamine (DA), tyramine, and tryptamine (Shih et al, 1999).

PEA levels increased significantly in all brain regions but serotonin and dopamine levels were unchanged in MAO B knockout (KO) mice (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. The highest increase in PEA was found in the striatum in the absence of MAO B (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. An increase in MAO B level occurs with aging (Fowler et al., 1980; Kumar et al. 2004), indicative of its potential role in the cognitive decline associated with aging-related neurological diseases. 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). MAO B is predominantly expressed in glial cells (Levitt et al., 1982; Westlund et al, 1985), and 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), 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. 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 to avoid excluding small yet significant changes in gene expression. 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 four 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 five 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 the ratio represents the number of the DEGs that matched the reference database for the disorder as compared to the total number of reference genes in IPA database for the disorder. 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. IPA was used to cluster functionally related genes corresponding to specific canonical pathways to gain further insight into the underlying mechanism. 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. 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. 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 in 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 2α, 2β, and 1γ 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 MAO B 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 α, two β, and a γ subunit. There is a decrease in the expression of α2, α3, β1, β3, and γ3 subunits, and an increase in γ1 subunit in MAO B KO mice. 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. The high expression of GRIN2B is replaced by GRIN2A during normal development. There is no significant change in GRIN2A in the MAO B KO mice; 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. Memantine (Namenda®) is a GRIN antagonist approved for Alzheimer’s disease and in Phase III trials for OCD and ALS (unlike cycloserine, which is a GRIN agonist). 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. 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. The α subunit can function on its own unlike the β subunit. 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). 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.

Our finding that the DEGs that function in brain injury or inflammation were upregulated in MAO B knockout mice is consistent with the prior reports. The earlier studies have shown that neuroinflammation is closely associated with oxidative stress. The kynurenine pathway is implicated in immune regulation and neuroprotection. The immunomodulatory enzyme indoleamine-pyrrole 2,3-dioxygenase (IDO) catalyzes the oxidation of L-tryptophan to N-formylkynurenine. The level of IDO-1 protein and the downstream metabolite was significantly increased in hypoxic human neuronal SH-SY5Y cells expressing MAO A but not MAO B (Lam et al., 2017). Tryptophan 2,3-dioxygenase (TDO) also catalyzes the oxidation of L-tryptophan to N-formyl-L-kynurenine. SH-SY5Y was originally derived from the human neuroblastoma SK-N-SH cell line. The expression of TDO protein (as well as IDO protein) and the catabolite in SK-N-SH cells was previously documented (Guillemin et al., 2007).

The reduced expression of the DEGs that function as gamma-aminobutyric acid (GABA) receptors in MAO B knockout mice suggests that the expression patterns of the GABA receptors and MAO B may correlate. A potential mechanism underlying the above correlation came from the finding that repressor element-1 silencing transcription factor (REST) suppresses the transcription of the GABA receptor subunit beta-3 (GABRB3) (Rouillard et al., 2016) as well as the MAO A gene (Lin et al., 2017). As MAO A and B genes arose via gene duplication, REST may also suppress MAO B expression.

Consistent with the above correlation, the transcript levels of both MAO B and GABRA2 were increased in the ventral tegmental area of the brain, which is involved in the regulation of motivations, emotions and affective disorders, in the mice that have been conditioned to trigger mixed anxiety or depression-like state (Galyamina, et al. 2017). Furthermore, western blot analysis performed on postmortem cerebella documented the increased level of GABRA2 protein in subjects with bipolar disorder and upregulated expression of GABRA2 protein and GABRG3 protein in individuals with major depressive disorder (Fatemi et al., 2013). Multiple reports have documented the overexpression of MAO B protein in the postmortem brain tissue of patients with major depressive disorder (Moriguchi et al., 2019; Klimek et al., 2003; Karolewicz et al., 2005). Monoamine oxidase inhibitors designed

to selectively suppress MAO B have been extensively used for the treatment of clinical depression (Finberg et al., 2016).

The gene-set based association analysis has shown that both GABRA3 and MAO B are associated with major depressive disorder (Lee et al., 2012). Furthermore, a systems-biology based machine learning approach was used to characterize individual variability in pain perception by integrating gene variant data with clinical data. The results showed that both MAO B and GABRA2 are associated with acute and chronic postoperative pain, which involves transcriptional dysregulation affecting multiple signaling pathways and sensitization throughout the pain neuraxis (Chidambaran et al., 2020).

Our DEG analysis has uncovered the altered expression of several transcription factors involved in adult neurogenesis in the MAO B knockout mice. Penumbra refers to cell damage that propagates from the infarct core to adjacent tissues following an ischemic stroke. The analysis of protein expression changes following the focal photothrombotic infarction in the rat cerebral cortex revealed the altered levels of Wnt signal transduction pathway proteins and monoamine oxidase B protein involved in neurodegeneration or neuroprotection (Uzdensky et al., 2017).

5-HT_{1A} is a subtype of serotonin receptor involved in neuromodulation that affects anxiety, mood, aggression and other neurological processes. Its transcription is negatively regulated by Hes5 (Albert et al., 2014), and the overrepresentation of Hes5 in major depressive disorder has been described (Savitz et al., 2009).

Paired box gene 6a (PAX6) is a highly conserved homeobox-containing transcription factor that assumes a critical role of balancing the differentiation and proliferation of cortical neural progenitor cells. The expression level of PAX6 was downregulated in the neural progenitor cells generated from induced pluripotent stem cells derived from bipolar disorder patients (Madison et al., 2015). Genetic analysis has shown that MAO B is associated with bipolar disorder (Lin et al., 2000).

Pitt–Hopkins syndrome is a neurological disorder exhibiting a range of symptoms including intellectual disability and delayed motor or cognitive movement. The autism spectrum disorder is caused by the haploinsufficiency of TCF4, a transcription factor containing a basic helix-loop-helix motif (Sweatt, 2013). The missense mutation A614V destabilizes the protein, reducing the level of TCF4 protein (Sepp et al., 2012). Previously, it was shown that mice deficient in MAO A display autistic behaviors (Bortolato et al., 2018). Specific genetic variants of MAO B with diminished protein activity increase serotonin and the susceptibility to autism in human males (Chakraborti et al., 2016).

Our DEG analysis in MAO B knockout mice has uncovered the altered expression of multiple sodium channel subunits that are commonly involved in Parkinson's disease, Alzheimer's disease, autism, bipolar affective disorder, OCD and ALS.

The neurodegenerative disease Parkinson's disease is characterized by a progressive decline in cognitive function. Dopamine is oxidized by MAO B to generate reactive oxygen species, which may contribute to the loss of dopaminergic neurons. The depletion of dopamine

is accompanied by neuroinflammation. The persistent activation of microglia expressing sodium channels may promote neurodegeneration by secreting inflammatory molecules and cytokines.

Various MAO B inhibitors have been developed to treat Parkinson's disease. Among them is Zonisamide that alleviates Parkinson's disease symptoms through inhibiting MAO B, which is expressed in astrocytes of the central nervous system. Zonisamide also reduces the sodium channel subunit $Na_v1.6$ protein, which is expressed in the microglia in the post-mortem brain of Parkinson's disease patients (Hossain et al., 2018). The neuroprotective role of Zonisamide results from inhibiting MAO B as well as $Na_v1.6$ involved in neuroinflammation. The results are consistent with our finding that the SCN8A gene, which encodes $Na_v1.6$, was downregulated in MAO B knockout mice. MAO B deficient mice are resistant to the Parkinsongenic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Shih et al., 1999).

The expression of $Na_v1.1$ protein along with $Na_v1.6$ protein was elevated in the hippocampus, which is critical for learning and memory, following the dopamine depletion in the rat model of Parkinson's disease. Treatment with the sodium channel blocker phenytoin improved cognitive deficits (Wang et al., 2019). Consistently, both the SCN1A gene encoding $Na_v1.1$ and SCN8A gene encoding $Na_v1.6$ were downregulated in MAO B knockout mice resistant to the Parkinsongenic neurotoxin.

Inflammation may play a significant role in the pathogenesis of multiple neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis (Glass et al., 2010). Microglia are the major immune cells that survey the microenvironment within the brain and uncontrolled inflammation may lead to the production of factors detrimental to the brain. Multiple reports have linked microglial dysfunction to the altered neuronal networks in Alzheimer's disease patients.

Impaired microglial functions were observed with increasing amyloid beta plaque deposition in the murine model of Alzheimer's disease (Krabbe et al., 2013). Both $Na_v1.1$ and $Na_v1.6$ proteins are expressed in microglia and the potential use of sodium channel blockers to treat epileptic symptoms of Alzheimer's disease is being explored (Shaikh et al., 2014).

The mutation in amyloid precursor protein is associated with a greater risk of developing seizures in Alzheimer's disease. The alteration in voltage-gated sodium channels has been implicated in hippocampal hyperactivity and seizures associated with Alzheimer's disease. $Na_v1.6$ protein represents a critical determinant of neuronal hyperexcitability in murine primary hippocampal neurons treated with amyloid-β peptide. Reducing $Na_v1.6$ protein through RNA interference reverted symptoms in hippocampal neurons of Alzheimer's disease-related Tg2576 mice (Ciccone et al., 2019).

The above results are consistent with our finding that the SCN8A gene encoding $Na_v1.6$ was downregulated in MAO B knockout mice. Previous works have documented the increase in MAO B in addition to the amyloid-β peptide in Alzheimer's disease (Schedin-Weiss et al., 2017). MAO B inhibitors have been used as anti-Alzheimer's disease agents to reduce stress caused by harmful side products of catalysis (Manzoor et al., 2020; Bortolato et al., 2008).

The sodium channel $Na_v1.6$ protein was upregulated in activated microglia and macrophages in experimental autoimmune encephalomyelitis and multiple sclerosis, suggesting that it may facilitate the development of multiple sclerosis by modulating microglial inflammation. Sodium channel blockers conferred a protective effect on axons in the animal model of multiple sclerosis (Craner et al., 2005). Sufinamide is a selective inhibitor of MAO B, which also blocks the function of sodium channels. Sufinamide provided significant protection against neuronal deficit by suppressing the activation of microglia in the central nervous system (Morsali et al., 2013). Consistently, the SCN8A gene, which encodes $Na_v1.6$, was downregulated in MAO B knockout mice.

Epilepsy is a neurological condition marked by periodic unprovoked seizures that involve hyperexcitability of the neurons of the central nervous system. Voltage-gated sodium channels play a critical role in initiating and propagating action potential and their abnormal expression may lead to neural hyperexcitability. A seizure-dependent increase in $Na_v1.6$ protein was observed in reactive astrocytes in the ipsilateral hippocampus of post-status epilepticus rats during the latent stage of epileptogenesis (Zhu et al., 2016). The antiepileptic drug phenytoin, which blocks sodium channels, was shown to reduce the number of microglia (Pappalardo et al. 2016). Further, mutations in the SCN2A gene encoding $Na_v1.2$ protein have been linked to neonatal epilepsy (Liao et al., 2021), and SCN2A was downregulated in MAO B knockout mice.

Multiple reports implicate the dysfunction of the sodium channel $Na_v1.7$ in neuropathic pain. $Na_v1.7$ protein is expressed in sympathetic neurons and nociceptive neurons, and mutations in the SCN9A gene that affect $Na_v1.7$ function modulated the severity of pain (Dib-Hajj et al., 2007; Fertleman et al., 2006). Specific molecules that block $Na_v1.7$ have been developed to suppress peripherally expressed voltage-gated sodium channels and relieve chronic pain (McGowan et al., 2009). Previously, MAO B was suggested to play a role in the development of neuropathic pain and postoperative pain (Villarinho et al., 2012), and the expression of SCN9A encoding $Na_v1.7$ was altered in MAO B knockout mice.

The reactive oxygen species generated by monoamine oxidases such as MAO B, which is expressed in mouse myocardium, were shown to cause cardiac oxidative stress (Kaludercic et al., 2011). Genetic variants of voltage-gated sodium channels associated with increased risk for cardiac arrhythmias have been identified. Specific mutations in the SCN1B gene, which change the $Na_v\beta.1$ polypeptide sequence to alter channel gating, have been linked to cardiac arrhythmia susceptibility (Watanabe et al., 2008; Watanabe et al., 2009). Distinct mutations in the SCN1B gene have been associated with epilepsy (Scheffer et al., 2007). A mutation in the SCN3B gene, which affects $Na_v\beta.3$ protein, has been linked to ventricular fibrillation (Valdivia et al., 2009). These genetic associations are supported our observation that the expression of SCN1B and SCN3B genes was altered in MAO B knockout mice.

4. Experimental Procedures

4.1 Microarray analysis

RNA was isolated from the brain tissue of MAO B KO (2-month old mice; equivalent to 20 human years) 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™ Mouse Gene 1.0 ST Array (Cat. No. 901168, Thermo-Fischer Inc., Waltham, MA, USA). Partek® Genomics Suite (PGS version 7, Partek Inc., St. Louis, MO, USA) was used for statistical analysis of microarray data to identify differentially expressed genes between MAO B KO and WT mice (n=5) of the same genetic background (Grimsby et al., 1997). 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. All procedures performed in studies involving mice were approved by Institutional Animal Care and Use Committee (#10159) at the University of Southern California.

4.3 Computational analysis of protein functions

The DEG data were further analyzed with QIAGEN's Ingenuity® Pathway Analysis (Cat. No. 830016, 830052, QIAGEN Redwood City, Inc., Redwood City, USA), which integrates the microarray data with known disease pathways to create a map of gene expression related to neurological diseases.

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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

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Article Highlights

- First study to show differentially expressed genes in 2-month old MAO B KO mice
- Altered genes are involved in brain injury, inflammation and neurogenesis
- Psychiatric and neurological disorder linked genes are altered in MAO B KO mice
- MAO B KO mice as model for developing drugs and studying neurodegenerative disease

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.

The altered genes linked to attention deficit hyperactive disorder, panic disorder, obsessive compulsive disorder, autism, amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, and bipolar affective disorder are displayed. RNA was isolated from the brain tissue of 2-month old MAO B KO and age-matched WT mice of the same genetic background and converted to cDNA to generate probes for hybridization. Gene expression levels were determined using Affymetrix GeneChip Array. Partek Genomics Suite was used for statistical analysis of microarray data to identify differentially expressed genes (DEGs) between MAO B KO and WT mice. DEGs are defined as MAO B KO vs WT [false discovery rate (FDR) < 0.05, fold-change (FC) >1.5]. Ingenuity Pathways Analysis (IPA) annotation database was used to identify DEGs linked to various neurological disorders.

Numerous altered genes are commonly related to these neurological disorders, indicating that there are overlapping molecular pathways.

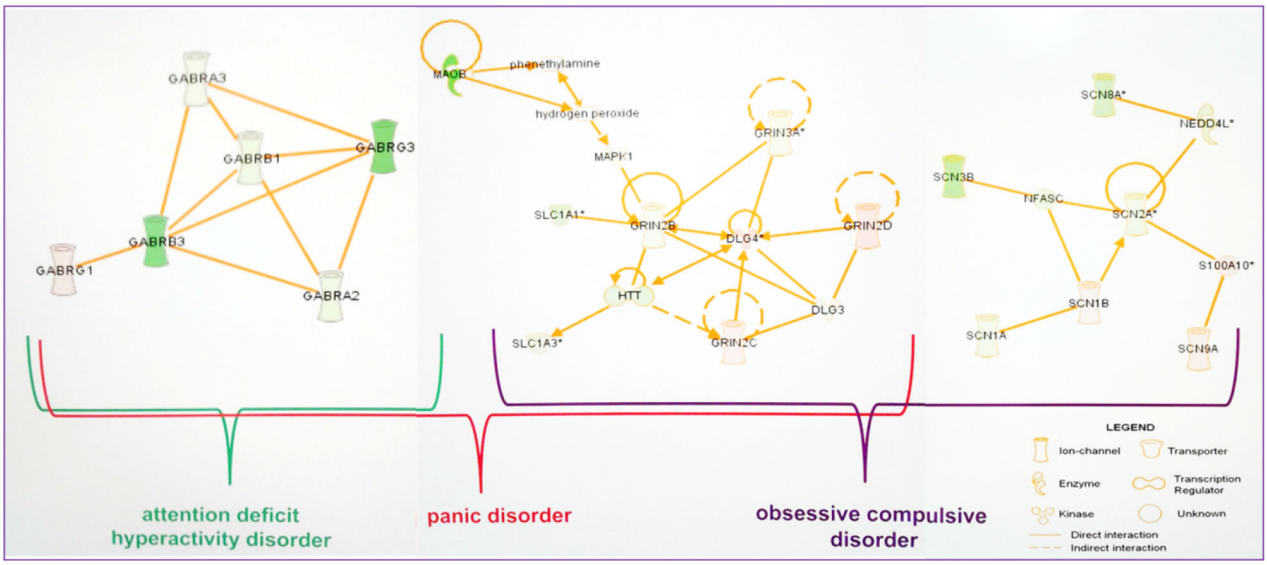

Figure 2. Gene interaction network map generated by Ingenuity Pathway Analysis (IPA) for altered genes in 2-month old MAO B KO mice.

The DEG data were further analyzed with IPA to integrate the microarray data with known disease pathways to generate a map of gene expression relevant to neurological diseases. The analysis shows the interaction network between molecules in the dataset. The interaction network map for attention deficit hyperactive disorder, panic disorder, and obsessive compulsive disorder is shown. The green color indicates decreased expression while the red color denotes increased expression. The intensity of color corresponds to fold-change. The lines connecting the GABA receptor (GABR) subunits indicate the protein-to-protein interactions.

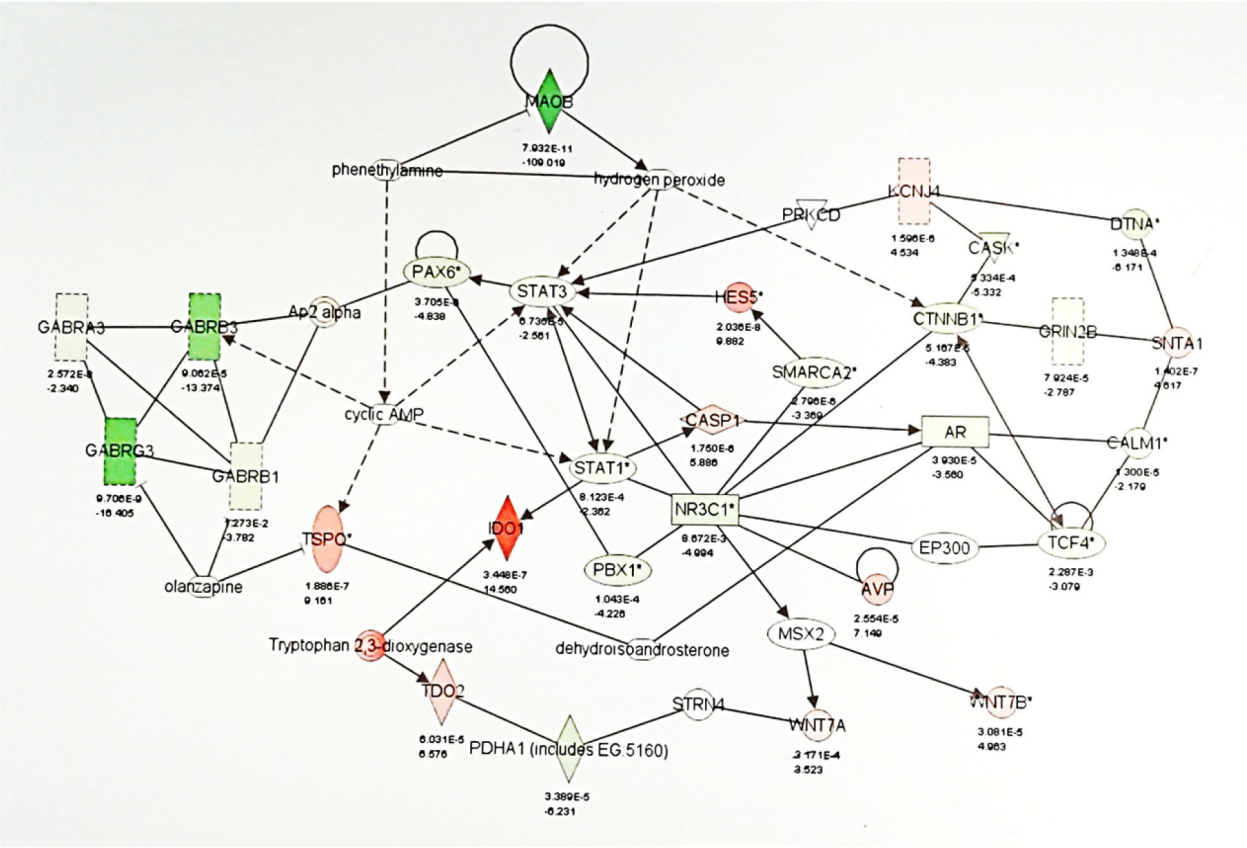

Figure 3. Regulatory networks and pathways generated by Ingenuity Pathway Analysis (IPA) for altered genes in 2-month old MAO B KO mice.

The IPA analysis revealed the potential pathways of upstream regulatory networks or downstream functions involving the DEGs. A network diagram depicting the possible signal transduction pathways involving inflammation-related genes, GABA receptors, and transcription factors pertinent to neuronal stem cells, which are listed in Tables 1 through 3, is shown. The other genes altered between the MAO B KO mice and WT mice were also included. Small molecules and other genes (without altered expression) were added to complete the pathway. The broken line indicates an indirect relation whereas the filled line denotes a direct protein-to-protein interaction.

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].

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 2

Decreased expression of GABA receptor subunits 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 3

Altered expression of transcription factors related to neurogenesis 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].

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