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## Adaptive and Behavioral Changes in Kynurenine 3-monooxygenase Knockout Mice: Relevance to Psychotic Disorders

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### Abstract

**BACKGROUND**—Kynurenine 3-monooxygenase (KMO) converts kynurenine to 3-hydroxykynurenine, and its inhibition shunts the kynurenine pathway - which is implicated as dysfunctional in various psychiatric disorders - towards enhanced synthesis of kynurenic acid (KYNA), an antagonist of both  $\alpha 7$  nicotinic acetylcholine and NMDA receptors. Possibly as a result of reduced KMO activity, elevated central nervous system levels of KYNA have been found in patients with psychotic disorders, including schizophrenia (SZ).

**METHODS**—In the present study, we investigated adaptive – and possibly regulatory – changes in mice with a targeted deletion of *Kmo* (*Kmo*<sup>-/-</sup>) and characterized the KMO-deficient mice using six behavioral assays relevant for the study of SZ.

**RESULTS**—Genome-wide differential gene expression analyses in the cerebral cortex and cerebellum of these mice identified a network of SZ- and psychosis-related genes, with more pronounced alterations in cerebellar tissue. KYNA levels were also increased in these brain regions in *Kmo*<sup>-/-</sup> mice, with significantly higher levels in the cerebellum than in the cerebrum. *Kmo*<sup>-/-</sup> mice exhibited impairments in contextual memory and spent less time than controls interacting with an unfamiliar mouse in a social interaction paradigm. The mutant animals displayed increased anxiety-like behavior in the elevated plus maze and in a light-dark box. After a D-amphetamine challenge (5 mg/kg, i.p.), *Kmo*<sup>-/-</sup> mice showed potentiated horizontal activity in the open field paradigm.

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**CONCLUSIONS**—Taken together, these results demonstrate that the elimination of *Kmo* in mice is associated with multiple gene and functional alterations that appear to duplicate aspects of the psychopathology of several neuropsychiatric disorders.

### Keywords

anxiety; cognition; kynurenic acid; locomotor activity; schizophrenia; social interaction

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### Introduction

Kynurenine 3-monooxygenase (KMO), an enzyme of the kynurenine pathway (KP) of tryptophan degradation, catalyzes the conversion of L-kynurenine (“kynurenine”) to 3-hydroxykynurenine (3-HK). 3-HK can both generate and scavenge reactive free radicals (1), and is also involved in modulating the neosynthesis of other neuroactive KP metabolites such as quinolinic acid (QUIN) and kynurenic acid (KYNA) (2) (Supplemental Figure 1). Impaired KMO function has been implicated in the pathophysiology of schizophrenia (SZ) (3–5), a major psychiatric disorder, which can be traced to abnormal brain development and is characterized by deficits in social and emotional functioning, thought disorder, abnormal perception of reality, and cognitive dysfunction (6). Specifically, postmortem data show that patients with SZ have lower mRNA levels of KMO and decreased KMO activity in the cerebral cortex (4, 7), though cortical 3-HK levels do not appear to be abnormal in SZ (8). Additionally, the non-synonymous single nucleotide polymorphism (SNP) rs2275163 in the gene encoding KMO - originally identified and cautiously linked to SZ by Aoyama and collaborators (9) – is associated with two established SZ endophenotypes, namely impairments in smooth pursuit eye movement and visuospatial working memory (7). In patients with bipolar disorder, a second polymorphism in the KMO gene (rs1053230) is associated with reduced KMO expression in the hippocampus and in lymphoblastoid cell lines, with higher cerebrospinal fluid (CSF) KYNA concentrations in individuals with a history of psychosis (3).

Dysfunctional KMO activity may, in fact, be *directly* related to the elevated levels of KYNA, which are also seen in the CSF and postmortem brains of patients with SZ and bipolar disorder (3, 8, 10–15). Thus, as demonstrated both after pharmacological KMO inhibition (16–18) and in mice with a genomic elimination of the *Kmo* gene (19), reduced KMO activity induces a shift in KP metabolism towards the pathway branch that produces KYNA (Supplemental Figure 1). Notably, after being released into the extracellular compartment, newly produced KYNA can act as an endogenous antagonist at  $\alpha 7$  nicotinic acetylcholine ( $\alpha 7$ nACh)(20) and N-methyl-D-aspartate (NMDA) receptors (21–23), both of which are critically involved in brain development (24) and cognition (25). However, KYNA may also target other recognition sites with less understood physiological significance (26, 27), and increased levels of endogenous KYNA at any of these sites may be related to the cognitive impairments seen in SZ. This link is supported by a considerable number of studies in rodents, which found that acute elevations of brain KYNA can induce cognitive dysfunctions, including deficits in sensorimotor gating (28, 29), working memory (30), contextual learning memory (31, 32), and cognitive flexibility (33).

The present study was designed to investigate possible changes in gene expression in the brain of mice with a targeted deletion of *Kmo* (*Kmo*<sup>-/-</sup> mice), to assess cerebral and cerebellar variations in KYNA levels in these mice (19), and to characterize the mutant animals behaviorally. Compared to *Kmo*<sup>+/+</sup> (wild-type) animals, *Kmo*<sup>-/-</sup> mice exhibited differential expression of several SZ- and psychosis-related genes and also showed significant impairments in cognition, social interaction, anxiety-like behaviors and D-amphetamine-induced locomotor activity. These findings support the existence of etiologically significant links between KP dysfunction and SZ, and, more generally, indicate the heuristic value of *Kmo*<sup>-/-</sup> mice for studying the pathophysiology of various psychiatric disorders.

## Materials and Methods

### Animals

Adult male *Kmo*<sup>-/-</sup> mice and *Kmo*<sup>+/+</sup> (wild-type) were bred on C57/BL6 or FVB/N backgrounds, as previously described (19) and detailed in Supplemental Materials.

### Microarray analysis

Whole genome gene-expression analysis was carried out on *Kmo*<sup>-/-</sup> and wild-type C57/BL6 mice as previously described (34, 35). Only differentially expressed transcripts with P<0.05 and >1.2-fold changes were included in subsequent analyses. Details are provided in Supplemental Materials.

### qPCR analyses

Experimental details for qPCR analyses are described in Supplemental Materials. The ratio of expression in *Kmo*<sup>-/-</sup> tissues compared to controls was calculated using the *ratibatch* function, with the mean Cp value of the two reference genes used as an internal control for each sample.

### Network and gene ontology analyses

Network analysis was performed using the STRING Database V10 (<http://string-db.org/>). (36) All 7 active prediction methods were employed for the analysis (Neighborhood, Gene Fusion, Co-occurrence, Co-expression, Experiments, Databases, Textmining), with a required confidence level of medium (0.400). An MCL clustering parameter of 2 was employed, and all disconnected nodes were removed, as well as nodes within small networks that did not form part of the major network identified. STRING was also used for gene ontology analysis of enriched biological processes above genome background. Significantly enriched processes were sorted by Bonferroni corrected P-values, using a cutoff of 0.05.

### Enzyme activity and metabolite analyses

Brains from *Kmo*<sup>-/-</sup> and wild-type FVB/N mice were dissected into cerebellum and cerebrum and stored at -80°C. On the day of the analyses, tissues were thawed out and processed as previously described and detailed in Supplemental Materials.

## Behavioral testing

Experimentally naive *Kmo*<sup>-/-</sup> and wild-type (FVB/N) mice were used for each behavioral paradigm, as described in detail in Supplemental Materials.

## Statistics

All analyses were performed using Prism<sup>®</sup> 6 (GraphPad Software, Inc. La Jolla, CA, USA), or IBM SPSS Statistics 22 (IBM SPSS Inc., Chicago, IL, USA). Significance was set at  $P < 0.05$ . All assumptions of each test were checked prior to the analyses.

All data are reported as the mean  $\pm$  S.E.M. Statistical details for each experiment are provided in Supplemental Materials.

## Results

### Differential gene expression profiling identifies a network of SZ-related genes in *Kmo*<sup>-/-</sup> mice

In order to investigate the regulatory changes in the *Kmo*<sup>-/-</sup> mice, we performed an unbiased screen for differentially expressed genes (DEGs) using Illumina Expression BeadChips (MouseWG-6 v2.0). Gene profiling identified a number of DEGs in both cerebrum and cerebellum ( $P < 0.05$ ), with a fold change of 1.2 (Supplemental Tables 1 and 2). To visualize these expression changes and to compare the forebrain to the cerebellum, a hierarchical clustering map was developed (Supplemental Figure 2). Of the two samples, the cerebrum exhibited a greater number of DEGs in *Kmo*<sup>-/-</sup> mice, with a total of 120 DEGs (46 upregulated and 74 downregulated) (Supplemental Figure 2). In the cerebellum, a set of 24 genes was identified. Interestingly, there was an overlap of only 6 genes between forebrain and cerebellum: *CNIH4*, *FCER1G*, *LYPLAL1*, *MGST3*, *MYOC* and *SLC22A6*. Of greater interest in the context of the present study, both regions presented changes in several genes that have been implicated as dysfunctional in SZ (see Supplemental Table 3 for a comprehensive list of supporting references). Strikingly, upon further analysis, ~33% of the identified cerebellar DEGs were found to be SZ-associated, and these were particularly enriched amongst the upregulated genes (7/16, ~44%). Indeed, when a more stringent cut-off of 1.3-fold change was applied, ~63% of the upregulated cerebellar genes were linked to SZ. A more modest fraction of SZ-related genes was also identified amongst the cerebrum DEGs, with 28/120 (~23%) linked to the disease; however, these were more evenly distributed between up- and down-regulated categories.

To ascertain whether the identified DEGs interact in a common network, we performed network analyses. Amongst 138 proteins encoded by the differentially expressed genes in the *Kmo*<sup>-/-</sup> mice, a total of 105 protein-protein interactions were observed, which represents a significant ~2-fold increase over the 53 interactions expected ( $P = 1.45e-10$ ). We found a single robust interaction network containing 67 of the hits in several functional clusters (Figure 1a). This analysis supports the notion that a large proportion of the DEGs arising from genomic elimination of KMO activity – and linked to increased KYNA levels – act in a common network. Finally, we analyzed gene ontology (GO) Biological Processes terms associated with the DEGs, and found several significantly enriched biological processes –

including nervous system development and neurogenesis – which have been previously linked to SZ (Supplemental Table 4).

A subset of SZ-implicated DEG changes was assessed by qPCR, and a fold change of 1.4 for upregulated genes and 0.71 change for downregulated genes was selected as a cutoff for further analyses. In the cerebrum, five of six SZ-related DEGs were validated: *AVP*, *EGR2*, *COX8B*, *INDO*, and *ENPP2* (Figure 1b). In the cerebellum, two of the four genes – *TCF7L2* and *NRGN* – remained significantly upregulated when assessed by qPCR, whereas the DEG changes of two others, *DAOI* and *EGR3*, were not validated (Figure 1c). Taken together, the qPCR analyses supported the microarray data in general, and, specifically, confirmed a number of DEGs implicated in SZ.

### Brain KYNA levels are elevated in *Kmo*<sup>-/-</sup> mice

As we have previously characterized the biochemical profile of *Kmo*<sup>-/-</sup> mice in the cerebrum, in the present study we compared KP changes specifically in the cerebellum versus the cerebrum in *Kmo*<sup>-/-</sup> mice and wild-type mice. The activity of KMO (Figure 2a) and the amount of its enzymatic product 3-HK (Figure 2b) were drastically reduced in both cerebrum and cerebellum of *Kmo*<sup>-/-</sup> mice. Conversely, KYNA levels were significantly elevated in both brain tissues in *Kmo*<sup>-/-</sup> mice, and there was an unexpected higher increase in KYNA levels in the cerebellum than in the cerebrum (Figure 2c).

### Deficits in contextual memory in *Kmo*<sup>-/-</sup> mice

We next investigated the functional impact of KMO elimination using a hippocampus-mediated behavioral task, the passive avoidance paradigm (PAP). *Kmo*<sup>+/+</sup> and *Kmo*<sup>-/-</sup> did not differ significantly in approach latencies during the acquisition trial (Figure 3a). Twenty-four h later, in the retention trial, the avoidance latencies of wild-type animals were significantly higher than the approach latencies in the acquisition trial, signifying learning of the PAP. Conversely, the avoidance latencies of *Kmo*<sup>-/-</sup> mice were not significantly improved, suggesting a deficit in contextual memory. Additionally, the avoidance latencies of *Kmo*<sup>-/-</sup> mice were significantly shorter than those of wild-type animals (Figure 3a).

### Impairments in social interaction in *Kmo*<sup>-/-</sup> mice

To characterize social interaction, we tested *Kmo*<sup>+/+</sup> and *Kmo*<sup>-/-</sup> in the three-chamber social approach apparatus (Figure 3b). There was a significant interaction between genotype and test chamber ( $F_{(2,66)}=3.741$ ,  $P=0.029$ ). Both *Kmo*<sup>+/+</sup> and *Kmo*<sup>-/-</sup> mice spent more time in the chamber containing the stranger (*Kmo*<sup>+/+</sup>:  $393 \pm 21$  sec; *Kmo*<sup>-/-</sup>:  $348 \pm 26$  sec) than in the chamber containing the novel object (*Kmo*<sup>+/+</sup>:  $129 \pm 16$  sec; *Kmo*<sup>-/-</sup>:  $184 \pm 19$  sec) ( $P<0.001$ ). Compared to wild-type animals, *Kmo*<sup>-/-</sup> mice spent a lower percentage of total time with the stranger mouse versus the novel object, suggesting a deficit in social interaction (Figure 3b).

### *Kmo*<sup>-/-</sup> mice display anxiety-like behaviors

The elevated plus-maze, the light-dark box, and open field tests were used to assess anxiety-like behaviors in *Kmo*<sup>-/-</sup> mice. In the elevated plus-maze, *Kmo*<sup>-/-</sup> mice showed significant reductions in the percentage of time spent in the open arm (Figure 4a) and in the number of

entries into the open arms (Figure 4b) compared to *Kmo<sup>+/+</sup>* mice. In the light-dark box test, *Kmo<sup>-/-</sup>* mice spent significantly less time in the light compartment compared to their wild-type counterparts (Figure 4c) and made a decreased number of entries into the light compartment (Figure 4d). Furthermore, although *Kmo<sup>-/-</sup>* mice displayed comparable horizontal, rearing, and center activities as *Kmo<sup>+/+</sup>* mice in a general assessment of locomotion (Supplemental Figure 3), we observed a significant increase in the corner time of the *Kmo<sup>-/-</sup>* animals (Time:  $F_{(11, 462)}=3.50$ ,  $P<0.001$ ; Genotype:  $F_{(1, 42)}=7.704$ ,  $P<0.01$ ; Interaction:  $F_{(11, 462)}=3.60$ ,  $P<0.0001$ ; Figure 4e). Together, these data demonstrate increased anxiety-like behaviors in *Kmo<sup>-/-</sup>* mice compared to *Kmo<sup>+/+</sup>* mice.

### ***Kmo<sup>-/-</sup>* mice show enhanced locomotor response to D-amphetamine**

Acute administration of D-amphetamine (5 mg/kg, i.p.) produced increased horizontal activity compared to saline treatment and potentiated the increase in horizontal activity in *Kmo<sup>-/-</sup>* mice as compared to the *Kmo<sup>+/+</sup>* mice (Time:  $F_{(29, 1160)}=15.24$ ,  $P<0.0001$ ; Genotype:  $F_{(3, 40)}=35.55$ ,  $P<0.0001$ ; Interaction:  $F_{(87, 1160)}=17.88$ ,  $P<0.0001$ , Figure 5a). Central activity in *Kmo<sup>-/-</sup>* mice was enhanced by acute administration of D-amphetamine compared to the *Kmo<sup>+/+</sup>* mice (Time:  $F_{(29, 1160)}=6.416$ ,  $P<0.0001$ ; Genotype:  $F_{(3, 40)}=11.85$ ,  $P<0.0001$ ; Interaction:  $F_{(87, 1160)}=6.117$ ,  $P<0.0001$ , Figure 5b). Compared to *Kmo<sup>+/+</sup>* controls, *Kmo<sup>-/-</sup>* mice also displayed increased rearing activity and decreased corner time (Supplemental Figure 4).

### **Prepulse inhibition in *Kmo<sup>-/-</sup>* mice**

Basal startle magnitude did not differ between genotypes ( $P=0.35$ ; Supplemental Figure 5a), nor were there any differences in habituation to startle (data not shown). In the variable prepulse intensity block there was a significant increase in percent PPI with increasing prepulse level ( $F_{(2, 92)}=85.1$ ;  $P<0.0001$ ), and a main effect of genotype ( $F_{(1, 46)}=4.7$ ;  $P<0.05$ ). There was no interaction between genotype and prepulse level ( $F_{(2, 92)}=0.6$ ;  $P=0.58$ ; Supplemental Figure 5b). PPI was similarly disrupted in *Kmo<sup>+/+</sup>* and *Kmo<sup>-/-</sup>* mice following administration of D-amphetamine (2 mg/kg, i.p.) or MK-801 (0.15 mg/kg, i.p.) (data not shown).

## **Discussion**

The present study was designed to explore gene expression changes in *Kmo<sup>-/-</sup>* mice and to compare the behavioral phenotypes of the knock-outs with wild-type controls. Moreover, the mutant animals provided an opportunity to relate changes in these outcome measures to comparable phenomena in clinical populations with reported KP abnormalities, including patients with SZ or bipolar disorder (3, 4, 7, 8, 10, 11, 13, 15).

Among the SZ-related DEGs validated by qPCR, *NRGN*, *EGR2* and *AVP* have been repeatedly linked to distinct phenotypic manifestations that are associated with psychiatric diseases. For example, after its gene was found in a genome-wide screen to be strongly associated with SZ (37), neurogranin (*NRGN*) was shown to be a postsynaptic calmodulin-binding protein that is required for synaptic plasticity (38). The early response gene (*EGR*) family is noteworthy for containing several compelling SZ susceptibility genes (39), and

studies in forebrain-specific conditional *EGR2* mutant mice revealed that *EGR2* can act as an inhibitory constraint for certain cognitive functions (40). Arginine vasopressin (*AVP*) is critical for social interactions (41), its receptor gene is associated with emotional withdrawal, which is frequently observed in persons with SZ (42), and elimination of the *AVP* gene causes distinct cognitive abnormalities in rats (43). Notably, qPCR analysis did not validate D-amino acid oxidase (*DAAO*), another DEG associated with SZ pathophysiology (44).

The present study also identified a number of other interesting DEGs, including genes coding for several proteins that play major roles in neurotransmission, such as the AMPA2 ionotropic glutamate receptor and the potassium channels *KCNK6*, *KCNJ10*, *KCNK13* and *KCNE2*, and for proteins which are directly related to KP metabolism, namely *INDO* (indoleamine 2,3-dioxygenase 1) and the putative KYNA transporter *OAT1* (45). The possible functional and translational significance of these DEG findings, as well as their causal relationship to chronically elevated KYNA levels, require further elaboration. Notably, by performing network analysis of microarray data in *Kmo*<sup>-/-</sup> mice, we observed significant alterations in several networks known to be relevant for nervous system development and neurogenesis. Identification of these network impairments further supported our plan to assess translationally relevant behaviors in *Kmo*<sup>-/-</sup> animals.

In line with our previous, more detailed biochemical assessment of mice from the same colony (19), KMO activity was essentially eliminated in adult *Kmo*<sup>-/-</sup> animals, though some enzyme activity (<3% of wild-type) was measurable in 2/10 knockout mice, possibly due to very minor oxidative conversion of kynurenine to 3-HK. Thus, while the present study does not categorically rule out the existence of functional KMO isoforms and non-enzymatic production of 3-HK, the present results confirm that a single KMO accounts almost exclusively for the formation of 3-HK from kynurenine in mice. Because of its conceptual importance in the context of the present study, we also verified that the abolition of KMO was associated with a large reduction in cortical 3-HK levels and a substantial *increase* in cortical KYNA levels (19) but did not measure anthranilic acid levels, which are also significantly elevated in both periphery and brain of *Kmo*<sup>-/-</sup> animals (19) as well as in the serum of individuals with SZ (46). Unfortunately, we were not able to determine the brain levels of the KP metabolites 3-hydroxyanthranilic acid or cinnabarinic acid, which may play a role in the pathophysiology of SZ (47, 48), due to limits in assay sensitivity.

Adult *Kmo*<sup>-/-</sup> animals displayed abnormalities in hippocampus-dependent contextual memory, assessed in the PAP. These results are in line with the demonstration that elevated brain KYNA is associated with abnormalities in hippocampus-dependent learning and memory (30, 32, 49, 50). The hippocampus is richly endowed with  $\alpha$ 7nACh and NMDA receptors, two preferential targets of endogenous KYNA (26, 27, 51) which are critically important in learning and memory (25). Their inhibition by elevated KYNA may therefore be causally related to the contextual memory deficits seen in *Kmo*<sup>-/-</sup> animals.

Assessed using a three-chamber social approach task (52), *Kmo*<sup>-/-</sup> mice also showed a deficit in social interaction. This finding is in agreement with reports documenting impaired social behavior in rats after the administration of KYNA's brain-penetrable bioprecursor

kynurenine during early postnatal development or adolescence (53, 54). Of note in this context, a deficit in social interactions is also seen in inbred BTBR T+tf/J mice, which likely have a compromised *Kmo* gene and display an array of autism-like behavioral phenotypes (55).

In line with previous studies demonstrating an increase in anxiety-like phenotypes after acute or repeated systemic kynurenine administration in rodents (56–58). *Kmo*<sup>-/-</sup> mice also displayed increased anxiety-like behavior when tested in three well-established experimental paradigms, i.e. the elevated plus maze, the light-dark box, and the open field. These effects were not associated with changes in spontaneous locomotor activity, which was examined in the open-field test. Notably, however, *Kmo*<sup>-/-</sup> mice showed an abnormally large increase in locomotor activity compared to wild-type mice when challenged with D-amphetamine. This heightened response is also seen in mice with experimentally induced chronic elevations in brain KYNA levels (56) and may be of relevance to the study of SZ, which is traditionally associated with dopaminergic hyperfunction in brain regions involved in motor behavior (59).

*Kmo*<sup>-/-</sup> mice did not display disruptions in PPI. In fact, the mutant animals showed slightly *more* inhibition to the prepulse than wild-type controls across increasing prepulse intensities. Also of note, no genotypic differences in PPI were observed when MK-801 (0.15 mg/kg) or D-amphetamine (2 mg/kg) were used as a provocative tool (60). While these results contrast with findings in healthy adult rats, which show disruptions in PPI when challenged acutely with kynurenine (28), our results in *Kmo*<sup>-/-</sup> mice are in line with findings showing that adult rats with chronically elevated levels of KYNA do not show PPI deficits (61). The mechanisms underlying the apparent different effects of chronic (i.e. life-long) and acute elevations of brain KYNA on gating (29), and implications for psychiatric diseases, where PPI disruptions are not always synonymous with cognitive deficits (62), are unclear and will need to be explored in future studies (see ref. 63 for further discussion).

In rodents, increases in brain KYNA inversely influence the extracellular concentrations of a number of major neurotransmitters, including glutamate, GABA, and dopamine (see ref. 64 for review). Alone or together, these effects, which are probably set in motion by the inhibition of  $\alpha 7$ nACh receptors (51), have been proposed to be responsible for the behavioral changes which are associated even with relatively modest increases in brain KYNA levels (28, 29, 32, 33, 49). The present study demonstrated that several of these behavioral phenomena, including deficits in cognition, impairment in social interaction, and anxiety-like phenotypes, are also seen in *Kmo*<sup>-/-</sup> mice, which have high brain KYNA levels. As these behavioral abnormalities are believed to be causally related to glutamatergic, GABAergic and/or dopaminergic mechanisms (65), it is tempting to speculate that neurochemical processes initiated by elevated KYNA play (a) distinct role(s) in the altered phenotypes we observed in the mutant animals.

The qualitative (DEGs) and quantitative (KYNA levels) differences between cerebellum and cerebrum of *Kmo*<sup>-/-</sup> mice deserve special attention. While the mechanisms underlying these differences remain to be clarified, several studies have indicated that the dynamics of cerebellar KP metabolism are distinct and likely developmentally regulated (66–69).



Notably, as intracerebellar infusions of nanomolar concentrations of KYNA cause remarkable changes in extracellular glutamate and dopamine in the distant prefrontal cortex (70), the disproportionately high KYNA levels in the cerebellum of *Kmo*<sup>-/-</sup> mice may account for some of the behavioral abnormalities we detected in these animals. Of significant interest in this context, the cerebellum is increasingly understood to play major roles in higher cognitive functions, and may be critically impaired in SZ (71).

In summary, our results indicate that *Kmo*<sup>-/-</sup> mice provide a heuristically useful experimental tool for studying the role of dysregulated KP metabolism in psychiatric disorders. As  $\alpha$ 7nACh and NMDA receptors, which likely serve as preferential targets of KYNA in the mammalian brain *in vivo* (26, 27), are critical to neurogenesis and play central roles in modulating neuronal migration and integration during brain maturation (72–76), we suspect that prolonged inhibition of these receptors, particularly during developmental periods, may be causally related to the behavioral phenotypes seen in adult *Kmo*<sup>-/-</sup> mice. Using biochemical, electrophysiological and behavioral outcome measures, experiments currently in progress in our laboratories are designed, *inter alia*, to investigate the impact of prenatal insults in both *Kmo*<sup>-/-</sup> and heterozygous (*Kmo*<sup>+/-</sup>) animals (77) and to evaluate genetic and pharmacological approaches to experimentally down-regulate brain KYNA levels in *Kmo*<sup>-/-</sup> mice (78, 79). However, caution is indicated when extrapolating studies with knock-out mice to pathological conditions in humans (80) and when assuming direct correlations between central and peripheral measures of KP metabolism (19). These studies will not only further define the heuristic value of using animals with targeted mutations of the KP to elucidate the etiology of SZ and other major psychiatric disorders, but may also shape new therapeutic strategies (2, 63, 81).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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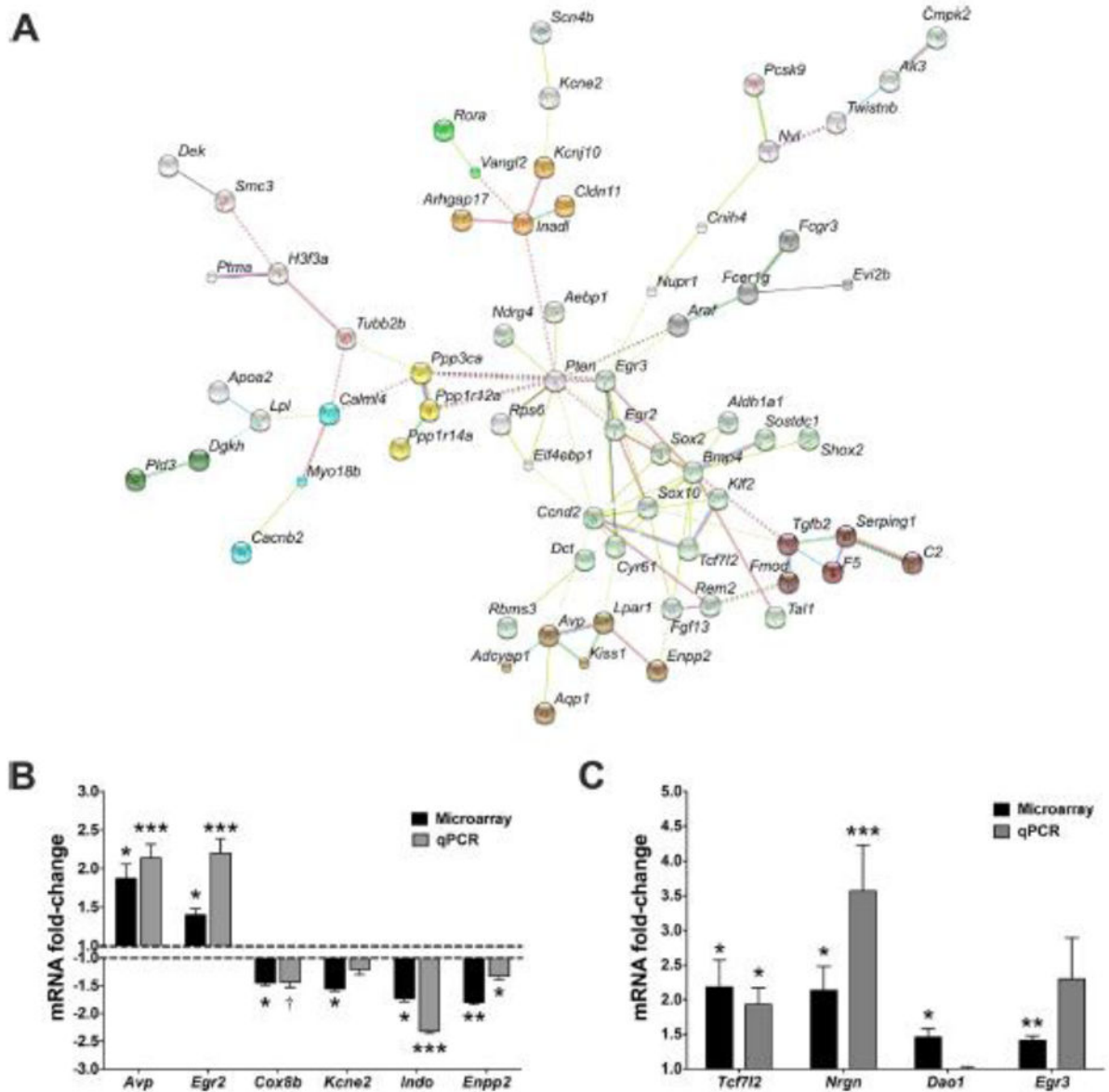
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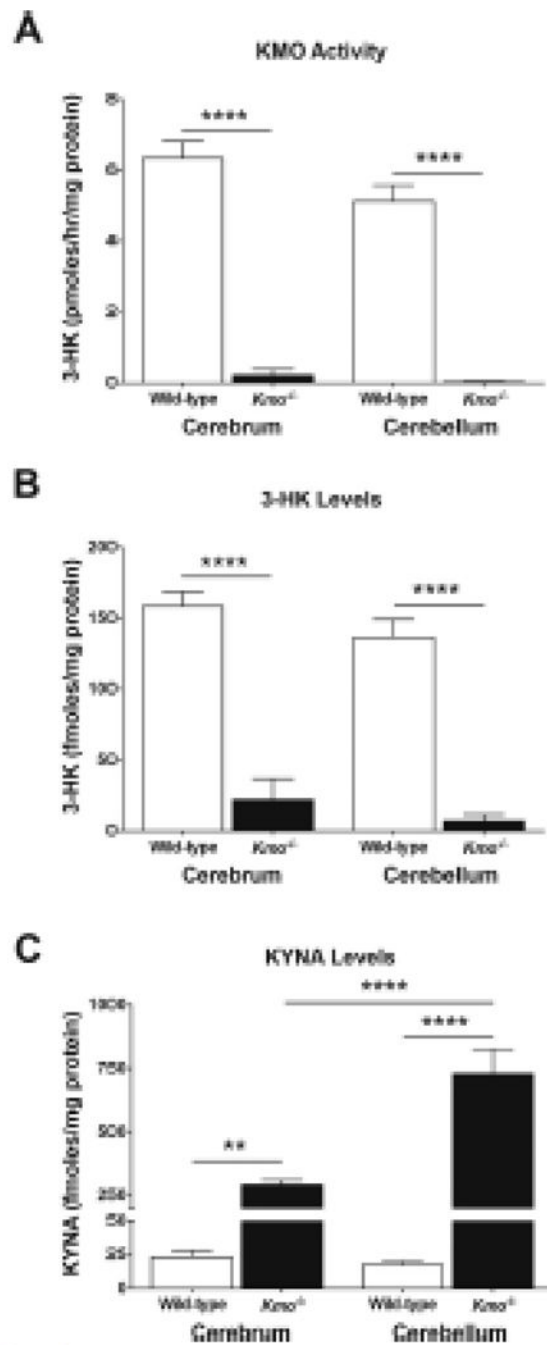
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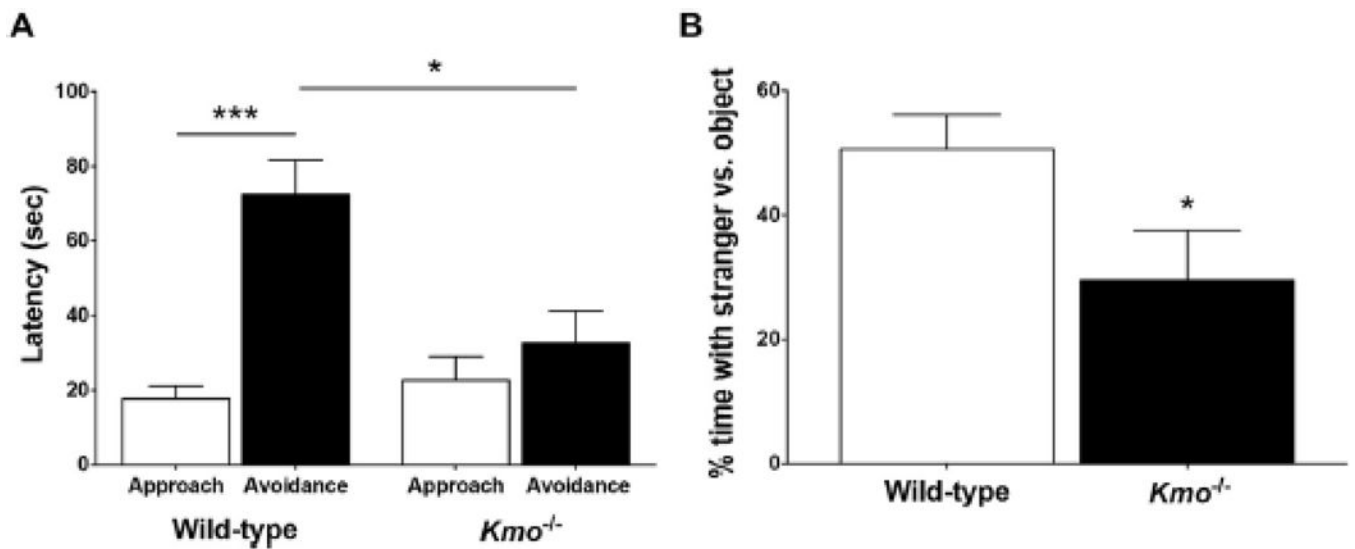


**Figure 1.** Regulatory gene changes in *Kmo*<sup>-/-</sup> mice. (A) Differentially expressed genes (DEGs) form a highly interconnected network. Network analysis determined the DEGs identified in *Kmo*<sup>-/-</sup> mice form a robust network containing 67/144 of the candidates. The network is characterized by several functional clusters highlighted with different colors. (B) qPCR validation of DEGs identified by microarray in the cerebral hemisphere. (C) qPCR validation of DEGs identified by microarray in the cerebellum. Data are mean ± SEM. †0.05 < P < 0.1; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001 compared to *Kmo*<sup>+/+</sup> mice. n=4-7 animals per group.

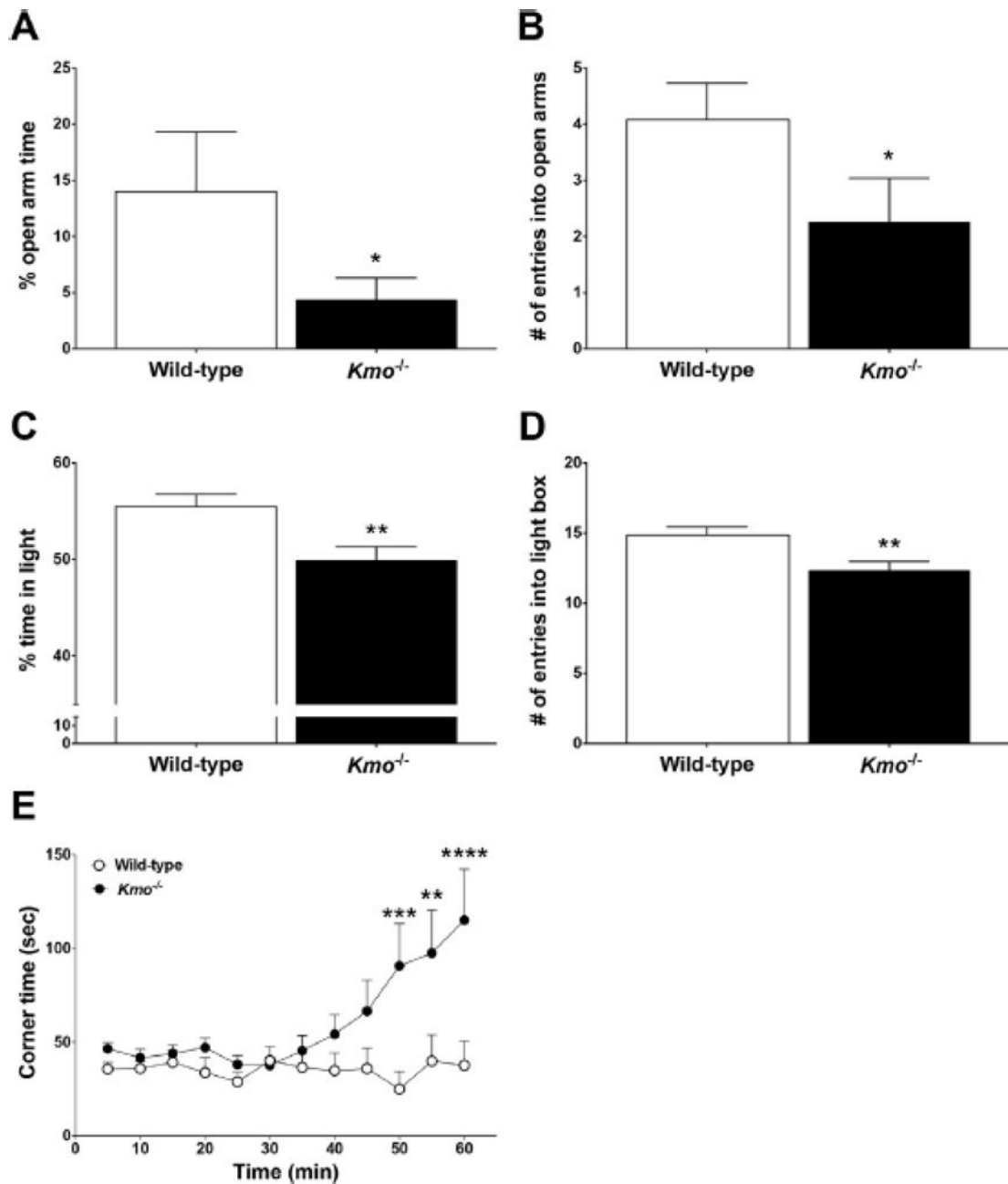


**Figure 2.** Kynurenine pathway metabolism in cerebrum and cerebellum of adult wild-type and *Kmo*<sup>-/-</sup> mice. (A) KMO activity is eliminated in both tissues in *Kmo*<sup>-/-</sup> mice. (B) Levels of 3-HK are reduced in both tissues in *Kmo*<sup>-/-</sup> compared to *Kmo*<sup>+/+</sup> mice. (C) Levels of KYNA are elevated in *Kmo*<sup>-/-</sup> mice. KYNA levels are significantly more elevated in the cerebellum than in the cerebrum. All data are the mean  $\pm$  SEM. \*\*P<0.01; \*\*\*P<0.001; n=8-10 per group.



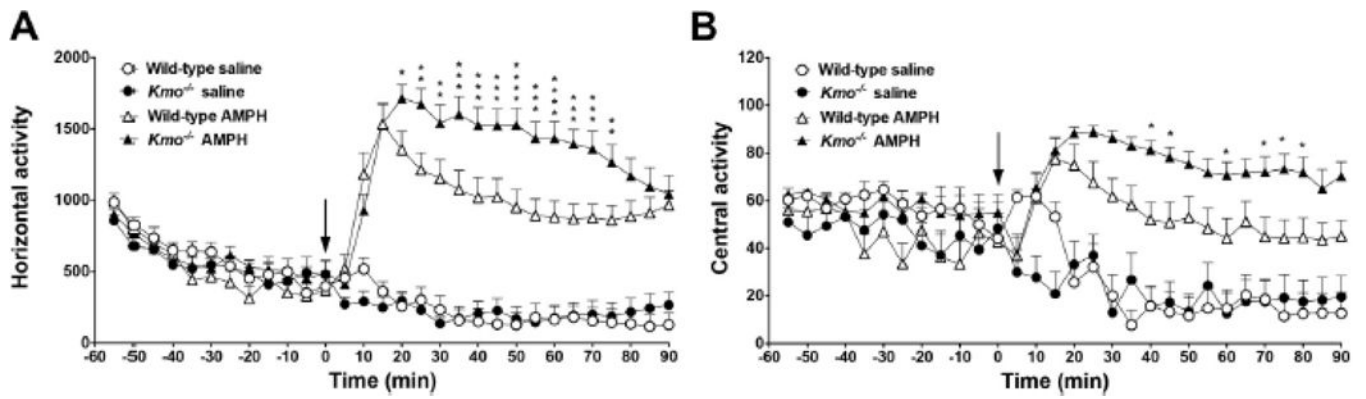


**Figure 3.** Contextual memory and social interaction. **(A)** Wild-type (n=14) and *Kmo*<sup>-/-</sup> (n=7) mice were tested in the passive avoidance paradigm. No genotypic difference in approach latency was observed on the training day. On Day 2, only *Kmo*<sup>+/+</sup> animals showed contextual memory, i.e. a significant difference between avoidance and approach latency. Avoidance latency differed significantly between wild-type and *Kmo*<sup>-/-</sup> animals. **(B)** Performance of wild-type (n=12) and *Kmo*<sup>-/-</sup> (n=12) mice in the three-chambered social interaction paradigm. Compared to *Kmo*<sup>+/+</sup> animals, mutant animals spent a lower proportion of time with the stranger mouse than with the novel object. All data are the mean ± SEM. \*P<0.05; \*\*\* P<0.001.



**Figure 4.**

Anxiety behavior in elevated plus maze (**A, B**), light-dark box (**C, D**), and open field (**e**). In the elevated plus maze, *Kmo*<sup>-/-</sup> mice (n=12) spent significantly less time in the open arm (**A**) and entered the open arms less frequently (**B**) than wild-type animals (n=12); In the light-dark box, *Kmo*<sup>-/-</sup> mice (n=17) spent significantly less time in the light compartment (**C**) and entered the light compartment less frequently (**D**) than wild-type mice (n=24); (**E**) In the open field, *Kmo*<sup>-/-</sup> mice (n=21) spent more time in the corners than wild-type animals (n=23). All data are the mean ± SEM. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001.



**Figure 5.**

Increased locomotor activity after D-amphetamine (AMPH; 5 mg/kg). At time 0 (arrows), animals received an i.p. injection of either AMPH (wild-type: n=12; *Kmo*<sup>-/-</sup>: n=11) or saline (wild-type: n=11; *Kmo*<sup>-/-</sup>: n=10). AMPH increased both horizontal (A) and central (B) activity significantly more in *Kmo*<sup>-/-</sup> mice than in wild-type animals. All data are the mean  $\pm$  SEM. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001 versus wild-type.