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Posted Date: 8 October 2025

doi: 10.20944/preprints202510.0580.v1

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Article

Behavioral Balance in Tryptophan Turmoil: Regional Metabolic Rewiring in Kynurenine Aminotransferase II Knockout Mice

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Abstract

Background: Cognitive, emotional, and social dysfunctions pervade neuropsychiatric disorders; dysregulated tryptophan (Trp)–kynurenine signaling, notably kynurenic acid (KYNA) from kynurenine aminotransferases (KATs), is implicated in Alzheimer’ disease, Parkinson’s disease, depression, and post-traumatic stress disorder (PTSD), among others. In novel CRISPR/Cas9-generated KAT II knockout (*aadat*^{−/−} aka. *kat2*^{−/−}) mice, we observed despair-linked depression-like behavior with peripheral excitotoxicity and oxidative stress. KAT II’s role and its crosstalk with serotonin, indole-pyruvate, and tyrosine (Tyr)–dopamine remain unclear. It is unknown whether deficits extend to cognitive, emotional, motor, and social domains or whether brain tissues mirror peripheral stress. **Objectives:** Delineate domain-wide behaviors, brain oxidative/excitotoxic profiles, and pathway interactions attributable to KAT II. **Results:** Behavior was unchanged across strains. *kat2*^{−/−} deletion remodeled Trp metabolic pathways: 3-hydroxykynurenine increased, xanthurenic acid decreased, KYNA fell in cortex and hippocampus but rose in striatum, quinaldic acid (QAA) decreased in cerebellum and brainstem. Such spatially restricted shifts delineate metabolic stress as

a core transdiagnostic liability, converging with increased oxidative burden and amplified excitotoxic mechanisms. **Conclusion:** Here we show *kat2* deletion reshapes regional Trp metabolism while reinforcing despair-linked emotional bias, this study highlights novel metabolic signatures as stratification biomarkers. These insights may foster double-hit animal models to dissect the convergence of depression and PTSD, ultimately informing targeted therapeutic approaches.

Keywords: tryptophan; kynurenine; serotonin; dopamine; kynurenine aminotransferase; oxidative stress; excitotoxicity; gut microbiota; transgenic mice; behavioral test

1. Introduction

Cognitive dysfunction, emotional dysregulation, motor impairment, and atypical social behavior represent core clinical features across a broad spectrum of neuropsychiatric and neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, schizophrenia (SCZ), and autism spectrum disorder (ASD) [1–5]. As the incidence of these conditions continues to rise globally, their cumulative impact on public health infrastructure, caregivers, and society becomes increasingly profound [2,3,6–8]. These growing challenges underscore the urgent need for elucidating the molecular and cellular mechanisms that drive these complex disorders [1,4,9,10]. Among the neurobiological systems under investigation, the metabolism of tryptophan (Trp)—an essential amino acid and a biochemical precursor to numerous neuroactive compounds—has garnered substantial attention in recent years [9,11–14].

Trp metabolism plays a pivotal role in modulating central nervous system (CNS) functions, particularly those related to cognitive abilities, mood regulation, and social behavior [15–19]. Dysregulation within these metabolic pathways has been increasingly linked to the pathophysiology of diseases marked by cognitive decline and deficits in social functioning [15–17,20,21]. The kynurenine (KYN) pathway is the principal route for Trp catabolism, accounting for approximately 90% of total metabolic flux [15,16,21–23] (Figure 1). This pathway produces a variety of bioactive metabolites with diverse effects on CNS function [15,16,18,24,25]. Among these, kynurenic acid (KYNA) stands out due to its ability to modify excitatory neurotransmission through its action on multiple receptors, including N-methyl-D-aspartate (NMDA), α 7-nicotinic acetylcholine, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors [24,26–30]. KYNA is synthesized via the irreversible transamination of KYN by kynurenine aminotransferase enzymes (KATs), with the KAT II isoform being particularly prominent in the brain [19,25,28,31,32]. In contrast, another KYN pathway metabolite, 3-hydroxykynurenine (3-HK), contributes to neurotoxicity by promoting oxidative stress through the generation of reactive oxygen species [17,20,22,33,34]. While historically KYNA and 3-HK were categorized as strictly neuroprotective and neurotoxic, respectively, emerging evidence reveals more complex, context-dependent functions that vary based on concentration, receptor expression patterns, and disease-specific factors [15,24,33,35,36].

In addition to the KYN pathway, several alternative routes for Trp metabolism significantly influence CNS homeostasis [15,37–40]. One such pathway is the 5-HT–melatonin (MEL) system [15,37,40–42]. 5-HT, synthesized from Trp, is a critical neurotransmitter involved in mood regulation, affective balance, and social cognition. Its downstream metabolite, MEL, regulates circadian rhythms and sleep architecture—factors integrally linked to learning, memory consolidation, and executive functioning [39,42–44]. Perturbations in this pathway are associated with a wide range of psychiatric disorders, including major depressive disorder (MDD), generalized anxiety disorder (GAD), and disturbances in sleep and circadian regulation [12,39,41,45,46]. Here, we quantify regional 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) to estimate serotonergic turnover in vivo and relate these indices to KYN-pathway shifts.

Another prominent route is the indole–pyruvate pathway, primarily driven by the gut microbiota [47–51]. In this pathway, microbial enzymes convert Trp into several indole derivatives

through distinct enzymatic reactions [47–49,51,52]. These indole metabolites are capable of crossing the intestinal barrier and influencing the CNS by modulating neuroinflammatory processes, maintaining gut epithelial integrity, and regulating blood-brain barrier (BBB) permeability [47,48,51,53,54]. This bidirectional communication along the gut-brain axis has been implicated in the pathophysiology of mood disorders, ASD, and other conditions characterized by social and emotional dysregulation [48,52,55–57]. We therefore profile brain-region levels of indole-3-acetic acid (IAA) and indole-3-carboxaldehyde (ICA) as sentinel markers of gut-brain indole signaling in KAT II knockout mice.

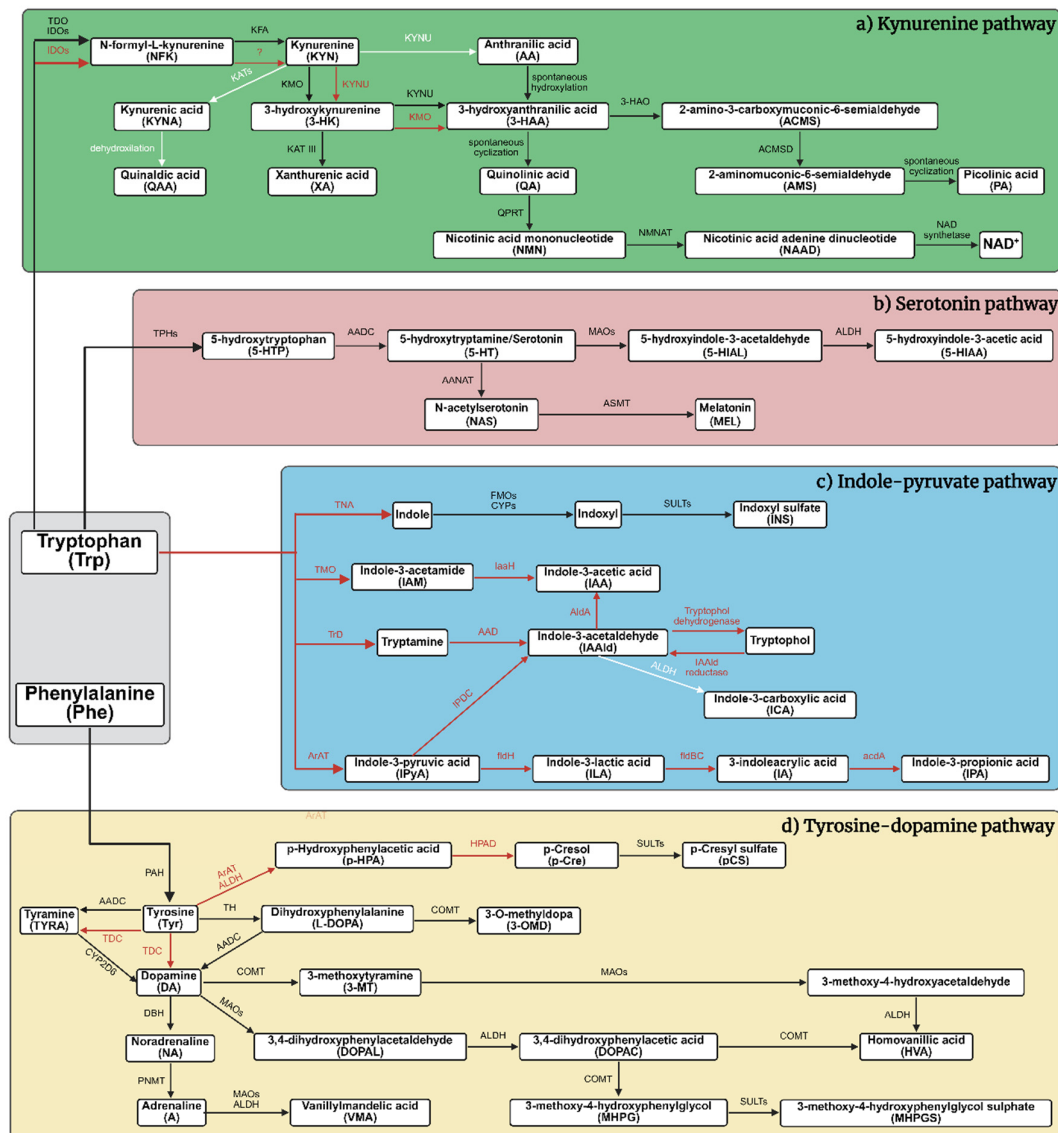


Figure 1. Host-microbiota co-metabolism of aromatic amino acids: tryptophan (Trp) and phenylalanine routes to neuroactive and redox-active metabolites. a) Kynurenine pathway (green): tryptophan enters the kynurenine axis via TDO/IDO to N-formyl-L-kynurenine → kynurenine, branching through KMO to 3-hydroxykynurenine and 3-hydroxyanthranilic acid (→ quinolinic acid) or through KATs to kynurenic acid. 2-amino-3-carboxymuconic-6-semialdehyde cyclizes/oxidizes toward quinolinic acid, which is converted by QPRT → nicotinic acid mononucleotide and onward to NAD⁺ (via NMNAT/NAD synthetase). This pathway balances neurotoxic (3-hydroxykynurenine, quinolinic acid) and neuroprotective (kynurenic acid) signals while supplying cellular NAD⁺. b) Serotonin pathway (rose): tryptophan is hydroxylated by TPHs to 5-

hydroxytryptophan and decarboxylated by aromatic L-amino acid decarboxylase (AADC) to serotonin. Serotonin is catabolized by MAOs/ALDH to 5-hydroxyindole-3-acetic acid, or acetylated/methylated by AANAT → N-acetylserotonin and HIOMT/ASMT → melatonin. This route links gut/brain serotonin tone with circadian signaling. c) Indole-pyruvate pathway (blue; microbiota–host): bacterial TNA converts tryptophan to indole, which is oxidized to indoxyl and sulfated in the host to indoxyl sulfate. Parallel microbial transamination/reduction/oxidation steps yield indole-3-pyruvic acid → indole-3-lactic acid/indole-3-acetic acid, indole-3-acetaldehyde, 3-indoleacrylic acid, and indole-3-propionic acid. These ligands engage AhR, fortify epithelial barriers, and shape systemic immunity. d) Tyrosine (Tyr)–dopamine pathway (yellow): phenylalanine → tyrosine (PAH) → levodopa (TH) → dopamine (AADC) → noradrenaline (DBH) → adrenaline (PNMT), with COMT/MAOs/ALDH producing 3,4-dihydroxyphenylacetic acid, 3-methoxytyramine, homovanillic acid, vanillylmandelic acid, and 3-methoxy-4-hydroxyphenylglycol. In parallel, microbial fermentation of phenylalanine/tyrosine generates p-Hydroxyphenylacetic acid and p-Cresol, further host-conjugated to p-Cresyl sulfate—an impactful uremic/toxic metabolite. Black arrows: the host routes; red arrows: the gut microbiota routes; white arrows: host and microbiota routes with the same enzyme. 3-HAA, 3-hydroxyanthranilic acid; 3-HAO, 3-hydroxyanthranilate oxidase; 3-HK, 3-hydroxykynurenine; 3-MT, 3-methoxytyramine; 3-OMD, 3-O-methyl dopa; 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HIAL, 5-hydroxyindole-3-acetaldehyde; 5-HT, 5-hydroxytryptamine/serotonin; 5-HTP, 5-hydroxytryptophan; A, adrenaline; AA, anthranilic acid; AAD, amino acid decarboxylase; AADC, aromatic L-amino acid decarboxylase; AANAT, arylalkylamine N-acetyltransferase; acdA, acyl-CoA dehydrogenase; ACMS, 2-amino-3-carboxymuconic-6-semialdehyde; ACMSD, amino-β-carboxymuconate-semialdehyde-decarboxylase; AldA, indole-3-acetaldehyde dehydrogenase; ALDH, aldehyde dehydrogenase; AMS, 2-aminomuconic-6-semialdehyde; ArAT, aromatic amino acid aminotransferase; ASMT, acetylserotonin-O-methyltransferase; COMT, catechol-O-methyltransferase; CYP2D6, cytochrome P450 2D6; CYPs, cytochrome P450 monooxygenases; DA, dopamine; DBH, dopamine β-hydroxylase; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPAL, 3,4-dihydroxyphenylacetaldehyde; fldBC, phenyllactate dehydratase; fldH, indole-3-pyruvate ferredoxin oxidoreductase; FMOs, flavin-containing monooxygenases; HPAD, 4-hydroxyphenylacetate decarboxylase; HVA, homovanillic acid; IA, 3-indoleacrylic acid; IAA, indole-3-acetic acid; IaaH, indole-3-acetamide hydrolase; IAALd, indole-3-acetaldehyde; IAM, indole-3-acetamide; ICA, indole-3-carboxylic acid; IDOs, indoleamine 2,3-dioxygenases (IDO1 and IDO2); ILA, indole-3-lactic acid; INS, indoxyl sulfate; IPA, indole-3-propionic acid; IPDC, indole-3-pyruvate decarboxylase; IPyA, indole-3-pyruvic acid; KAT III, kynurenine aminotransferase III; KATs, kynurenine aminotransferases (KAT I, II, III, and IV); KFA, kynurenine formamidase; KMO, kynurenine-3-monooxygenase; KYN, kynurenine; KYNA, kynurenic acid; KYNU, kynureninase; L-DOPA, dihydroxyphenylalanine/levodopa; MAOs, monoamine oxidases (MAO-A and MAO-B); MEL, melatonin; MHPG, 3-methoxy-4-hydroxyphenylglycol; MHPGS, 3-methoxy-4-hydroxyphenylglycol sulphate; NA, noradrenaline; NAD⁺, nicotinamide adenine dinucleotide; NAAD, nicotinic acid adenine dinucleotide; NAS, N-acetylserotonin; NFK, N-formyl-L-kynurenine; NMN, nicotinic acid mononucleotide; NMNAT, nicotinamide mononucleotide adenylyltransferase; p-Cre, p-Cresol; p-HPA, para-hydroxyphenylacetic acid; pCS, p-Cresyl sulfate; PA, picolinic acid; PAH, phenylalanine hydroxylase; Phe, phenylalanine; PNMT, phenylethanolamine N-methyltransferase; QA, quinolinic acid; QAA, quinaldic acid; QPRT, quinolate phosphoribosyl transferase; SULTs, sulfotransferases; TDC, tyrosine decarboxylase; TDO, tryptophan-2,3-dioxygenase; TH, tyrosine hydroxylase; TMO, tryptophan-2-monooxygenase; TNA, tryptophanase; TPHs, tryptophan hydroxylases (TPH1 and TPH2); Trd, tryptophan decarboxylase; Trp, tryptophan; Tyr, tyrosine; TYRA, tyramine; VMA, vanillylmandelic acid; XA, xanthurenic acid; ?, unknown.

Additionally, Trp metabolism exerts regulatory effects on dopaminergic neurotransmission via its influence on the tyrosine (Tyr)–dopamine (DA) pathway [37,58–61]. Specifically, Trp availability impacts the synthesis of tetrahydrobiopterin (BH₄), a critical cofactor required by tyrosine hydroxylase—the rate-limiting enzyme in DA production [37,58,59,62,63]. Given DA's fundamental role in mediating reward processing, attentional control, and social engagement, this intersection further emphasizes the extensive reach of Trp metabolism in orchestrating complex behavioral and cognitive outcomes [39,59,64–66]. To capture this crosstalk, we quantify Tyr, levodopa (L-DOPA),

DA, and downstream metabolites alongside the pterin pool (BH4, dihydrobiopterin [BH2], and biopterin [BIO]).

In earlier research, we examined the interrelationships between affective disorders—such as MDD, GAD, and post-traumatic stress disorder—and systemic alterations in Trp and its downstream metabolites in *kat2^{-/-}* mice model [67]. Although traditionally conceptualized as mood disorders, these conditions also encompass profound cognitive impairments, including deficits in memory, sustained attention, and executive functioning [68–72]. Such impairments frequently manifest early in the course of illness and may persist independently of affective symptoms [68,70,72–74]. Notably, MDD has emerged as a significant risk factor for the subsequent development of neurodegenerative conditions like AD [68,70,75–77]. In parallel, individuals suffering from MDD and GAD often exhibit marked social dysfunction, including social withdrawal, blunted affect, and reduced empathic capacity [68,69,71,72,78]. These features closely parallel behavioral phenotypes observed in ASD and SCZ, further complicating differential diagnosis and therapeutic decision-making [68,71,78–81]. A mechanistic understanding of the molecular pathways that underlie these shared features is therefore of paramount importance [68,75,81–83].

To probe the contributions of Trp metabolic dysregulation to these behavioral phenotypes, we utilized a genetically engineered mouse model deficient in KAT II (*kat2^{-/-}*) [67]. This knockout model disrupts the biosynthetic pathway for KYNA, allowing for detailed investigation of downstream metabolic consequences. Targeted metabolomic profiling of urine and plasma revealed a pronounced decrease in KYNA levels accompanied by elevated concentrations of 3-HK. These findings reinforce the essential role of KAT II in modulating the balance between neuroprotective and neurotoxic metabolites within the KYN pathway. Building directly on this peripheral signature, the current study extends metabolomics to five brain regions (striatum [STR], cortex [CTX], hippocampus [HIP], cerebellum [CER], brainstem [STEM]) to resolve central, region-specific consequences of KAT II deletion.

Despite inherent limitations in modeling human psychiatric and neurodegenerative diseases in rodents—especially with regard to complex cognitive processes and nuanced social behaviors—this genetic model affords a valuable platform for dissecting the neurochemical substrates of behavior [84–88]. In the present study, we aimed to systematically evaluate cognitive function and social behavior in *kat2^{-/-}* mutant mice, with a particular focus on mapping these behavioral parameters to region-specific changes in the concentrations of Trp and its metabolites within the brain. This integrative approach offers a robust framework for uncovering potential neurochemical signatures that underlie cognitive and social impairments. Accordingly, our prespecified objectives were to (i) map region-resolved Trp metabolism across the KYN, 5-HT, and indole axes; (ii) quantify the Tyr to L-DOPA to DA cascade and its enzymatic and cofactor milieu (BH4, BH2, BIO); (iii) infer pathway activities using product-substrate ratios (for example, KMO and KAT fluxes, monoamine oxidase [MAO] and aldehyde dehydrogenase [ALDH] turnover) and derive oxidative-stress and excitotoxicity indices; (iv) link these neurochemical states to a broadened behavioral battery encompassing cognition (novel object recognition, object-based attention, Y-maze), motor coordination (rotarod), emotion (marble burying), and sociability (three-chamber); and (v) test concordance between brain and peripheral metabolic signatures. By coupling this expanded behavioral panel with multi-region neurochemical profiling, we aim to delineate how KAT II loss reshapes a KYN-tilted, cofactor-constrained, and indole-modulated milieu, and to determine whether such biochemical disequilibria necessarily generalize to global cognitive or social dysfunction, thereby informing pathway-targeted therapeutic strategies across neuropsychiatric spectra.

2. Materials and Methods

2.1. Ethical Approval

The Department of Nature Conservation of the Ministry of Agriculture authorized the use of genetically modified organisms in a level 2 biosafety closed system (permit number: TMF/43-

20/2015). The import of genetically modified animals was approved by the Department of Biodiversity and Gene Conservation of the Ministry of Agriculture (permit number: BGMF/37-5/2020). The investigations were conducted in accordance with the Ethical Codex for Animal Experiments and were approved by the Ethics Committee of the Faculty of Medicine at the University of Szeged, as well as by the National Food Chain Safety Office, under permission number CS/I01/00084-2/2025 and XI./1008/2025.

2.2. Animals

The C57BL/6N wild-type (WT) strain was originally sourced from Charles River Germany. The *kat2^{-/-}* strain was provided by our collaboration partners at Kyushu University (Fukuoka, Japan). A comprehensive description of the generation of the genetically modified strain can be found in our previously published article [67]. The animals were housed in groups of 4-5 per cage in polycarbonate enclosures (530 cm² floor area) under specific pathogen-free conditions at the Animal Facility of the Department of Neurology, University of Szeged. Environmental parameters were stringently controlled, with ambient temperature maintained at 24 ± 1 °C and 45-55% relative humidity under a 12:12 hours light:dark cycle. Throughout the duration of the investigation, mice had unrestricted access to standard rodent food and water.

2.3. Genotyping with Taqman Allelic Discrimination Assay

All animals were genotyped in advance, ensuring verification before the subsequent experimental studies. Mice were anesthetized with 2% isoflurane, and following local analgesia with 5% lidocaine ointment, a 3 mm fragment of the tail was excised using sterile instruments under aseptic conditions. Tissue samples were stored at -80 °C until further processing. For DNA extraction, 75 µl of a freshly prepared lysis buffer containing equal volumes of 25 mM NaOH and 0.2 mM disodium EDTA was added to each sample. After incubation at 95 °C for 30 minutes, the suspension was cooled to 4 °C and then neutralized with 75 µl of 40 mM TRIS-HCl buffer. This method was adapted from the HotSHOT protocol and consistently yields DNA suitable for reliable genotyping, as previously demonstrated by Truett et al.. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (MaestroGen, Taipei, Taiwan). The resulting DNA extracts were stored at -20 °C until analysis [89].

Genotyping was performed using a fluorescence-labeled TaqMan allelic discrimination assay. The forward primer sequence was 5'-TAACAGTGCATCCCGAGTGA-3', the reverse primer sequence was 5'-GAGGGCTCTGGCTTTGTTTT-3', while probe 1 and probe 2 sequences were 5'-6-FAM-CAACGAGCCTGGCCAGAA-BHQ-1-3' and 5'-HEX-TGCAACGACTGGCCAGAAAG-BHQ-1-3', respectively (Metabion, Steinkirchen / Planegg, Germany). For each reaction, the PCR was assembled using the following reagents: PCR Master Mix (5 µl; PCR Biosystems, London, UK), forward primer (1 µl), reverse primer (1 µl), probe 1 (0.5 µl), probe 2 (0.5 µl), DNA template (1 µl), and water (1 µl). Non-template control reactions contained water instead of DNA. Reaction mixtures were aliquoted into 96-well plates. PCR amplification and allelic discrimination were performed in single-plex reactions using a CFX Opus 96 Real-Time PCR System (Bio-Rad Laboratories, Hercules, California, USA) according to the manufacturer's instructions. The amplification protocol consisted of an initial denaturation at 95 °C for 10 min, followed by 40 cycles of 92 °C for 15 sec and 60 °C for 1 min. Fluorescence data were analyzed with CFX Maestro software.

This approach ensured that all mutant animals included in the experiments were confirmed to carry the targeted genetic modification, and wild-type mice were correctly identified as controls.

2.4. Behavioral Tests

Cognitive, emotional, motor, and social domains were assayed with novel object recognition for recognition memory, Y-maze spontaneous alternation for working memory, marble burying test (MBT), the accelerating rotarod for motor coordination, and the automated three-chamber task for

sociability. The behavioral experiments were performed on 8-week-old male mice of the C57BL/6N and *kat2^{-/-}* strains, with n = 8-12 animals included per group. To minimize stress, the animals were habituated to handling by the experimenters for one week prior to testing. To ensure comparability of the results, all tests were conducted between 8:00 a.m. and 12:00 p.m. Prior to testing, animals were transferred to the experimental laboratory one hour in advance, allowing sufficient time for acclimatization to the environmental conditions. All test sessions were recorded using a video tracking system (Basler ace Classic acA1300-60 gm, Basler AG, Ahrensburg, Germany) in combination with behavioral analysis software (EthoVision XT14, Noldus Information Technology BV, Wageningen, the Netherlands).

2.4.1. Novel Object Recognition Test (NORT)

The Novel Object Recognition Test (NORT) has become an established and widely employed approach for investigating various phases of learning and non-spatial object memory in rodents [90–94]. For the NORT, behavioral assessments were conducted in a 60 x 60 x 60 cm open-field arena. Three distinct objects—different in color and shape but matched in size and scale relative to the animals—were utilized. The test was carried out across three consecutive days. On the habituation day, each animal was placed in the empty arena for a duration of 10 minutes to facilitate acclimatization to the new environment. On the second day (training session), animals were allowed to explore two of the three objects for 10 minutes, enabling the encoding of object-related information. Animals that failed to exhibit any interaction with the object designated as the familiar object during the training phase were excluded from further analysis in the experiment. On the third day (test session), one of the familiar objects from the training phase was substituted with the previously unencountered third object. This unfamiliar item functioned as the novel object, whereas the remaining object served as the familiar object for the purpose of evaluating recognition memory. During NORT we measured the following parameters: 1) time spent with the training object in training phase, 2) time spent with the familiar object in training phase, 3) time spent with the familiar object in testing phase, 4) time spent with the novel object in testing phase.

During both NORT and OBAT, the duration of investigation directed toward the novel and familiar objects was systematically recorded. To assess object recognition and novelty preference, two normalized metrics were employed: the discrimination index (DI) and the preference index (PI). The DI quantifies the relative preference for the novel object compared to the familiar one, while the PI expresses the proportion of total exploration time that the animal allocated to the novel object. Both indices account for individual variability in total investigation time and were computed using the following formulas (Eq. 1, Eq. 2).

$$\text{discrimination index (DI)} = \frac{T_{\text{novel}} - T_{\text{familiar}}}{T_{\text{novel}} + T_{\text{familiar}}} \tag{1}$$

$$\text{preference index (PI)} = \frac{T_{\text{novel}}}{T_{\text{novel}} + T_{\text{familiar}}} \times 100 \tag{2}$$

2.4.2. Object-Based Attention Test (OBAT)

Attention deficits constitute a hallmark feature of cognitive dysfunction commonly observed across various neuropsychiatric conditions. The object-based attention test (OBAT), originally developed by Wulaer and colleagues [95–99], represents a validated behavioral paradigm for evaluating attentional performance in rodents. This method is conceptually analogous to object-based visual attention assessments employed in human cognitive research. Similar to the NORT, the OBAT leverages the rodent’s intrinsic exploratory drive and preference for novelty. The experimental setup comprises a two-compartment arena with dimensions of 40 x 40 x 40 cm (larger compartment) and

20 x 40 x 40 cm (smaller compartment), and utilizes six distinct objects. These objects differ in color and shape but are comparable in size. The procedure consists of two sequential phases: a training phase and a testing phase. During the training phase, the animal is introduced into the larger compartment containing five distinct objects and is allowed a 3-minute exploration period to become familiar with them. Animals that did not engage in any interaction with the object assigned as the familiar object during the training phase were excluded from subsequent experimental analysis. Subsequently, one of these previously encountered objects, along with a sixth, novel object is placed in the smaller compartment for the 3-minute test phase. The novel item serves as the novel object, while the reintroduced item functions as the familiar object. Healthy animals typically exhibit a clear preference for the novel object, indicative of intact attentional and recognition processes. Animals that did not engage with the object later serving as the familiar stimulus during the training phase were systematically excluded from subsequent experimental evaluation.

The following parameters were assessed during the NORT: 1) duration of interaction with the training objects during the training phase, 2) duration of interaction with the familiar object during the training phase, 3) time spent exploring the familiar object during the testing phase, and 4) time spent interacting with the novel object during the testing phase.

2.4.3. Y-Maze Test

The Y-maze represents a widely utilized paradigm for evaluating short-term memory function in rodents. Spontaneous alternation behavior—considered a robust index of spatial working memory—can be assessed by permitting animals unrestricted access to all three arms of the apparatus [100–103]. This exploratory pattern is guided by the rodent’s innate tendency to investigate novel, previously unvisited environments. Rodents exhibiting intact working memory capacity, and thereby preserved prefrontal cortical function, are capable of recalling which arms have been recently explored and display a preferential inclination of enter the arm that has not been visited in the most recent sequence. At the onset of the trial, the animal is positioned at the distal end of the longest arm of the Y-maze, oriented toward the central zone. Thereafter, it is granted an eight-minute period to freely explore the maze. The spontaneous alternation rate was determined according to the following formula (Eq. 3).

$$\text{spontaneous alternation (\%)} = \frac{\text{number of spontaneous alternations}}{\text{total number of arm entries} - 2} \times 100 \tag{3}$$

During the Y-maze test, we measured spontaneous alternation behavior as well as the total number of entries into all three arms.

2.4.4. Marble Burying Test (MBT)

The marble burying test (MBT) is primarily employed to evaluate compulsive and anxiety-related behaviors, especially in models of generalized anxiety and obsessive-compulsive disorder [104–108]. Nonetheless, certain studies suggest that it may also be used indirectly to assess aspects of social behavior and cognitive functioning, or to provide supplementary insights in these domains. Elevated levels of compulsive behavior are frequently accompanied by social deficits. In animal models exhibiting impaired sociability, intensified marble burying may reflect increased behavioral rigidity and social withdrawal. Although MBT is not classified as a cognitive test, the presence of repetitive, inflexible actions may signal cognitive rigidity, potentially associated with dysfunctions in the frontal CTX.

The animals were individually placed in a transparent plastic arena measuring 40 x 24 x 18 cm. The base of the apparatus was filled with a 5 cm deep layer of fresh bedding material. To allow adequate ventilation while preventing escape. The enclosure was covered with a transparent plastic lid (40 x 24 cm, 1 cm thick) featuring six circular perforations, each 1 cm in diameter. At one end of the arena, sixteen glass marbles (each with dimensions of 1 x 1 x 1 cm³) were positioned on the

bedding in a regular square grid formation. The outermost marbles were placed 3.5 cm from the arena walls, with 5 cm spacing between adjacent marbles. Each mouse was allowed to explore the arena freely for a period of 30 minutes. The marbles were categorized based on their status: intact, displaced, partially buried (0-75%), or fully buried (75-100%). These measurements were subsequently analyzed and compared across experimental groups.

2.4.5. Three Chamber Test (3CT)

Deficits in reciprocal social interactions—characterized by diminished interest in conspecifics and impaired ability to maintain social engagement—constitute core features in the diagnostic criteria of social neuropsychiatric disorders, such as autism spectrum disorder and SCZ. Sociability is operationally defined as the propensity of the test mouse to spend a greater proportion of time in the compartment containing a novel conspecific, as opposed to the compartment housing a novel inanimate object. A supplementary and confirmatory metric involves the quantification of time spent engaging in olfactory investigation of the novel conspecific relative to the novel object, thereby providing an index of direct social interaction. Additionally, the number of transitions between compartments serves as an intrinsic control parameter for assessing exploratory locomotor activity [109–113].

A rectangular three-chambered apparatus was employed for the 3CT. Each compartment measured 20 x 40.5 x 22 cm. The chambers were divided by opaque gray plastic walls, each containing manually operated doors (7.5 x 5 cm) to allow controlled access between compartments. Cylindrical wire-mesh enclosures (15 cm in height, 7 cm in diameter) were positioned in both lateral chambers. The mesh structure, composed of bars spaced 1 cm apart, permitted adequate airflow between the interior and exterior of the cylinder while simultaneously preventing direct physical contact between the test subject and the stimulus animal or object placed within. This configuration allowed for the assessment of social preference and investigatory behavior while minimizing confounding factors related to tactile interaction [109,114–117]. The test protocol consisted of three distinct phases. During the habituation phase, the subject mouse was confined to the center chamber of the apparatus for a period of 10 minutes with all doors closed, allowing acclimatization to the environment. In the sociability phase, the doors to the lateral chambers were opened, and the test mouse, starting from the center chamber, was allowed to freely explore all three compartments for 10 minutes. One lateral chamber contained an empty wire-mesh enclosure, while the other housed a wire cage enclosing a novel conspecific that was matched to the test animal in sex, age, and body weight. The social novelty preference phase followed a similar structure: the subject animal started from the center chamber and was given 10 minutes to explore the entire apparatus. In this phase, the previously encountered conspecific from the sociability phase served as the familiar animal, while a non-familiar, sex-, age- and weight-matched conspecific was introduced into the formerly empty cage, serving as the novel animal.

During the sociability phase, we quantified 1) time spent in the social chamber, 2) time spent in the non-social chamber, 3) time spent in the center chamber, 4) time spent sniffing the social cage, 5) time spent sniffing the non-social cage, 6) number of entries to the social chamber, 7) number of entries to the non-social chamber, 8) number of entries to both chambers. In the social novelty preference phase, we measured the 1) time spent in the novel chamber, 2) time spent in the familiar chamber, 3) time spent in the center chamber, 4) time spent sniffing the novel animal's cage, 5) time spent sniffing the familiar animal's cage, 6) number of entries to the novel chamber, 7) number of entries to the familiar animal's chamber, 8) number of entries to both chambers.

2.4.6. Rotarod Test

The rotarod test is a widely established behavioral assay for evaluating motor coordination and balance in rodents. The classical accelerating rotarod paradigm primarily assesses maximal motor performance under progressively increasing demand [118–122]. The apparatus comprised a rotating rod equipped with an automated fall-detection system at the base, which interfaced with TSE

RotaRod V4.2.6 system (TSE Laboratory, Germany) to automatically terminate the timer upon the animal's fall. Animals underwent a two-day habituation and training protocol. On Day 1, each mouse was placed individually on the rotating rod, which was maintained at a constant speed of five revolutions per minute (rpm) for a duration of three minutes. Should the animal have fallen before the allotted time elapses, it was promptly repositioned on the rod. Following the initial session, the animal was returned to its home-cage, and the procedure was repeated twice more at 30-minute intervals. On Day 2, the training procedure was repeated, with the rotation speed increased to a constant 10 rpm, thereby introducing a higher motoric challenge and reinforcing task familiarity. The testing phase was conducted on Day 3. During this phase, animals were placed on the rod, which now accelerated linearly from 5 to 40 rpm over a 3-minute period. Each animal underwent three test trials, with a 30-minute inter-trial interval. Unlike during training, animals were not returned to the rod after falling. The latency to fall—defined as the time the animal remained on the rod before falling—was recorded automatically via the tracking software. The average latency across the three test trials served as the composite performance score for each subject, reflecting overall motor coordination and skill retention under increasing demands.

2.5. Ultra-High-Performance Liquid Chromatography with Tandem Mass Spectrometry (UHPLC-MS/MS)

2.5.1. Brain Samples

We used a randomized, blinded, region-resolved design that integrates targeted liquid chromatography with tandem mass spectrometry (LC-MS/MS) metabolomics with a standardized behavioral battery in *kat2* knockout and WT mice, and we report procedures according to ARRIVE 2.0 to maximize reproducibility. Regarding the brain samples, following the determination of tissue weights, the samples were homogenized using an ultrasonic homogenizer (UP100H, Hielscher Ultrasound Technology, Germany) set to 100% amplitude and a 0.5 cycle setting. Homogenization was performed in threefold volumes of ice-cooled LC-MS grade water relative to tissue mass (e.g., 90 μ l of water was added to 30.0 mg of tis-sue). We quantified Trp-KYN, serotonergic, and indole metabolites, plus catecholamine intermediates and selected cofactors, using previously published multiplex LC-MS/MS methods and protocols [123,124]. For PA, the MRM transition was 124.0 $\rightarrow$ 106.0 m/z, with a retention time of 1.21 min, using a declustering potential of 75 V and a collision energy of 13 V. For ICA, the MRM transition was 146.1 $\rightarrow$ 118.0 m/z, with a retention time of 12.40 min, using a declustering potential of 50 V and a collision energy of 19 V. For IPA, the MRM transition was 190.1 $\rightarrow$ 130.1 m/z, with a retention time of 13.00 min, using a declustering potential of 50 V and a collision energy of 19 V. For ILA, the MRM transition was 206.1 $\rightarrow$ 188.1 m/z, with a retention time of 12.00 min, using a declustering potential of 50 V and a collision energy of 13 V. For INS, the MRM transition was 211.9 $\rightarrow$ 131.9 m/z, with a retention time of 11.80 min, using a declustering potential of -50 V and a collision energy of -25 V. For pCS, the MRM transition was 186.9 $\rightarrow$ 107.0 m/z, with a retention time of 12.70 min, using a declustering potential of -50 V and a collision energy of -26 V. All reagents and chemicals were of analytical or liquid chromatography–mass spectrometry grade. Trp and its metabolites, and their deuterated forms: d4-serotonin, d5-tryptophan, d4-kynurenine, d5-kynurenic acid, d4-xanthurenic acid, d5-5-hydroxyindole-acetic acid, d3-3-hydroxyanthranilic acid, d4-picolinic acid, and d3-quinolinic acid were purchased from Toronto Research Chemicals (Toronto, ON, Canada). d3-3-hydroxykynurenine was obtained from Buchem B. V. (Apeldoorn, The Netherlands). Acetonitrile (ACN) was provided by Molar Chemicals (Halásztelek, Hungary). Methanol (MeOH) was purchased from LGC Standards (Wesel, Germany). Formic acid (FA) and water were obtained from VWR Chemicals (Monroeville, PA, USA). The UHPLC-MS/MS system consisted of a PerkinElmer Flexar UHPLC system (two FX-10 binary pumps, solvent manager, autosampler and thermostatic oven; all PerkinElmer Inc. (Waltham, MA, USA)), coupled to an AB SCIEX QTRAP 5500 MS/MS triple quadrupole mass spectrometer and controlled by Analyst 1.7.1 software (both AB Sciex, Framingham, MA, USA).

2.5.2. Plasma and Urine Samples

Plasma and urine samples were collected, prepared and measured according to previously published methodologies [67,123,124].

2.6. The Enzyme Activities of Tryptophan (Trp) Metabolism

The enzyme activity of Trp metabolism was estimated by calculating the ratio of the product-to-substrate concentration ratio.

2.7. Oxidative Stress and Excitotoxicity Indices

The oxidative stress index was derived by calculating the ratio between the concentration of the presumed pro-oxidant metabolite 3-HK and the combined concentrations of the putative antioxidant metabolites KYNA, anthranilic acid (AA), and xanthurenic acid (XA) (Eq. 4).

$$\text{Oxidative stress index} = \frac{[3 - \text{hydroxykynurenine}]}{[\text{Kynurenic acid}] + [\text{Anthranilic acid}] + [\text{Xanthurenic acid}]}$$

(4)

The excitotoxicity index was determined by computing the ratio of quinolinic acid (QA), an NMDA receptor agonist, to KYNA, an endogenous NMDA receptor antagonist (Eq. 5).

$$\text{Excitotoxicity index} = \frac{[\text{Quinolinic acid}]}{[\text{Kynurenic acid}]}$$

(5)

2.8. Statistical Analysis

We performed statistical analyses using IBM SPSS Statistics version 28.0.0.0 (IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed with the Shapiro-Wilk test, and Q-Q plots were additionally employed to evaluate whether two datasets originated from the same distribution.

In the statistical evaluation of the NORT, OBAT and Y-maze, 3CT and rotarod test, inter-strain comparisons were performed using the independent samples t-test for normally distributed data, whereas the non-parametric Mann-Whitney U test was employed in cases where the assumption of normality was violated. Intra-strain comparisons of individual parameters were carried out using the paired samples t-test when data conformed to a normal distribution, and the Wilcoxon signed-rank test was applied for non-normally distributed datasets.

For the MBT, a mixed ANOVA model was used, followed by the Tamhane post-hoc test.

Regarding the UHPLC-MS/MS measurements, the normality of the variables was checked using the Kolmogorov-Smirnov test and visually checked using quantile-quantile plots, and the equality of variances was examined using Welch's F-test. Outliers were identified using Grubbs's test. Comparisons between the two groups were conducted using an independent samples t-test.

Values $p < 0.05$ were considered statistically significant. Our data are reported as means $\pm$ standard deviations (SD) for all parameters and experimental groups.

3. Results

3.1. Behavioral Tests

3.1.1. Novel Object Recognition Test (NORT)

During the NORT, both WT and *kat2^{-/-}* animals spent significantly more time interacting with the novel object compared to the familiar one. However, no significant differences were detected between the strains (Figure 2, Table 1, Table 2).

Table 1. Behavioral performance in WT and *kat2^{-/-}* mice across multiple cognitive, social, and motor tests (NORT, OBAT, Y-Maze, MBT, 3CT, and Rotarod). Analyses revealed no significant inter-strain differences.

Behavioral test type	Number of animals (WT/ <i>kat2^{-/-}</i>)	Phase of the test	Parameter of the test	WT mean ± SD	<i>kat2^{-/-}</i> mean ± SD	p-value
NORT	12/12	Testing phase	Time spent with familiar object (s)	17.500 ± 8.635	18.556 ± 11.886	p < 0.839
			Time spent with novel object (s)	66.250 ± 59.461	72.444 ± 38.730	p < 0.596
			Discrimination index	0.455 ± 0.321	0.592 ± 0.141	p < 0.294
			Preference index	72.760 ± 16.032	79.604 ± 7.061	p < 0.293
OBAT	12/12	Testing phase	Time spent with familiar object (s)	15.362 ± 7.437	10.729 ± 6.786	p < 0.905
			Time spent with novel object (s)	19.747 ± 7.820	15.796 ± 6.372	p < 0.268
			Discrimination index	0.136 ± 0.239	0.239 ± 0.287	p < 0.428
			Preference index (%)	56.802 ± 11.943	61.944 ± 14.355	p < 0.428
Y-maze	12/12	-	Spontaneous alternations (%)	52.833 ± 27.996	66.500 ± 18.880	p < 0.175
			Number of total entries	15.583 ± 11.579	18.000 ± 15.788	p < 0.954
MBT	10/13	-	Buried marbles	5.467 ± 4.207	6.133 ± 4.121	p < 0.738
			Partially buried marbles	4.267 ± 2.344	4.533 ± 2.326	p < 0.757
			Displaced marbles	1.733 ± 1.870	1.333 ± 1.345	p < 0.731
			Intact marbles	4.533 ± 3.248	4.000 ± 2.976	p < 0.643
3CT	12/12	Testing sociability	Time in social chamber (s)	265.717 ± 40.368	260.658 ± 54.993	p < 0.799
			Time in non-social chamber (s)	247.748 ± 25.751	247.988 ± 56.056	p < 0.989
			Time in center chamber (s)	86.536 ± 32.148	91.355 ± 28.005	p < 0.699
			Sniffing social cage (s)	145.955 ± 39.690	136.336 ± 37.149	p < 0.546
			Sniffing non-social cage (s)	114.603 ± 33.637	117.447 ± 33.452	p < 0.837
			Total sniffing time (s)	260.558 ± 38.784	253.783 ± 47.129	p < 0.704
			Social chamber entries (number)	12.667 ± 3.725	13.417 ± 4.621	p < 0.666
			Non-social chamber entries (number)	13.167 ± 4.174	12.833 ± 4.687	p < 0.855
			Total entries (number)	25.833 ± 7.673	26.250 ± 9.245	p < 0.905

Testing novelty preference			Time in novel chamber (s)	263.188 ± 60.124	253.058 ± 68.641	p < 0.704
			Time in familiar chamber (s)	238.687 ± 55.961	237.654 ± 56.502	p < 0.964
			Time in center chamber (s)	98.126 ± 40.008	109.288 ± 53.470	p < 0.568
			Sniffing novel animal's cage (s)	129.261 ± 50.164	109.373 ± 44.085	p < 0.313
			Sniffing familiar animal's cage (s)	95.015 ± 51.306	92.903 ± 62.090	p < 0.928
			Total sniffing time (s)	224.276 ± 75.342	202.276 ± 79.270	p < 0.493
			Novel chamber entries (number)	9.917 ± 3.450	11.083 ± 3.679	p < 0.431
			Familiar chamber entries (number)	10.250 ± 3.279	11.167 ± 4.764	p < 0.589
			Total entries (number)	20.167 ± 6.548	22.250 ± 7.979	p < 0.492
Rotarod	12/12	-	Mean time spent on the rod	100.428 ± 35.017	89.708 ± 41.453	p < 0.501

Mean ± SD. 3CT, three-chamber test; MBT, marble burying test; NORT, novel object recognition test; OBAT, object-based attention test.

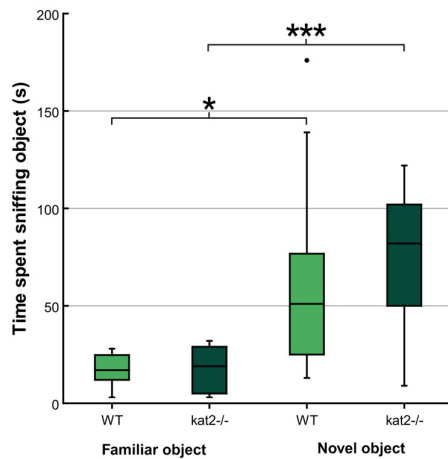
Table 2. Comparative behavioral performance of wild-type (WT) and *kat2^{-/-}* mice in object recognition (NORT, OBAT) and social interaction/novelty preference (3CT). In the NORT, both WT and *kat2^{-/-}* mice demonstrated a significant preference for the novel object. In the OBAT, the mutant strain exhibited greater exploration of the novel object. In the 3CT, both genotypes spent more time in the side chambers than in the center chamber during both the sociability and novelty preference phases.

Test type	Phase of the test		WT			<i>kat2^{-/-}</i>		
NORT	Testing phase		Sniffing familiar object (s)	Sniffing novel object (s)	p-value	Sniffing familiar object (s)	Sniffing novel object (s)	p-value
			17.500 ± 8.635	66.250 ± 59.461	p < 0.018 *	18.556 ± 11.886	72.444 ± 38.730	p < 0.001 ***
OBAT	Testing phase		Sniffing familiar object (s)	Sniffing novel object (s)		Sniffing familiar object (s)	Sniffing novel object (s)	
			15.362 ± 7.437	19.747 ± 7.820	p < 0.081	10.729 ± 6.786	15.796 ± 6.372	p < 0.039 *
3CT	Testing sociability		Time in social chamber (s)	Time in center chamber (s)		Time in social chamber (s)	Time in center chamber (s)	
			265.717 ± 40.368	86.536 ± 32.148	p < 0.001 ***	260.658 ± 54.993	91.355 ± 28.005	p < 0.001 ***
			Time in non-social chamber (s)	Time in center chamber (s)		Time in non-social chamber (s)	Time in center chamber (s)	
			247.748 ± 25.751	86.536 ± 32.148	p < 0.001 ***	247.988 ± 56.056	91.355 ± 28.005	p < 0.001 ***
			Time in social	Time in non-social		Time in social	Time in non-social	

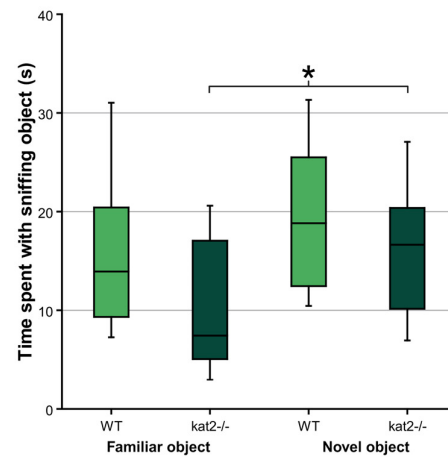
Testing novelty preference	chamber (s)		p < 0.319	chamber (s)		p < 0.691
	265.717 ± 40.368	247.748 ± 25.751		260.658 ± 54.993	247.988 ± 56.056	
	Sniffing social cage (s)	Sniffing non-social cage (s)		Sniffing social cage (s)	Sniffing non-social cage (s)	
	145.955 ± 39.690	114.603 ± 33.637	p < 0.110	136.336 ± 37.149	117.447 ± 33.452	p < 0.240
	Social chamber entries (number)	Non-social chamber entries (number)		Social chamber entries (number)	Non-social chamber entries (number)	
	12.667 ± 3.725	13.167 ± 4.174	p < 0.389	13.417 ± 4.621	12.833 ± 4.687	p < 0.089
	Time in novel chamber (s)	Time in center chamber (s)		Time in novel chamber (s)	Time in center chamber (s)	
	263.188 ± 60.124	98.126 ± 40.008	p < 0.001 ***	253.058 ± 68.641	109.288 ± 53.470	p < 0.002 **
	Time in familiar chamber (s)	Time in center chamber (s)		Time in familiar chamber (s)	Time in center chamber (s)	
	238.687 ± 55.961	98.126 ± 40.008	p < 0.001 ***	237.654 ± 56.502	109.288 ± 53.470	p < 0.001 ***
	Time in novel chamber (s)	Time in familiar chamber (s)		Time in novel chamber (s)	Time in familiar chamber (s)	
	263.188 ± 60.124	238.687 ± 55.961	p < 0.453	253.058 ± 68.641	237.654 ± 56.502	p < 0.754
	Sniffing novel animal's cage (s)	Sniffing familiar animal's cage (s)		Sniffing novel animal's cage (s)	Sniffing familiar animal's cage (s)	
	129.261 ± 50.164	95.015 ± 51.306	p < 0.109	109.373 ± 44.085	92.903 ± 62.090	p < 0.530
	Novel chamber entries (number)	Familiar chamber entries (number)		Novel chamber entries (number)	Familiar chamber entries (number)	
	9.917 ± 3.450	10.250 ± 3.279	p < 0.474	11.083 ± 3.679	11.167 ± 4.764	p < 0.681

For each paradigm, parameters include object sniffing duration, time allocation across chambers, sniffing of social/non-social targets, and chamber entries. Statistical significance was assessed for within-group contrasts (familiar vs. novel; social vs. non-social; chamber comparisons), and p-values are reported. Asterisks denote significance levels: *, p < 0.05, **, p < 0.01, ***, p < 0.001. 3CT, three-chamber test; *kat2^{-/-}*, kynurenine aminotransferase II knockout mice; NORT, novel object recognition test; OBAT, object-based attention test; WT, wild-type mice.

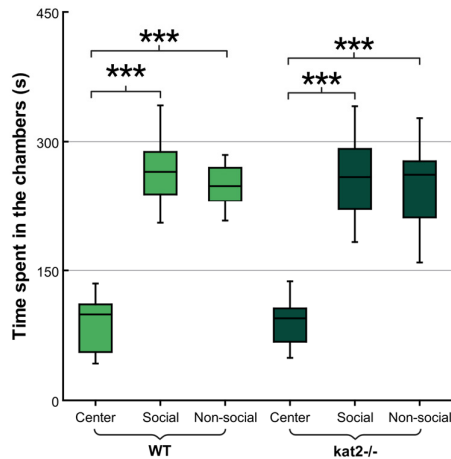
a) NORT - Time spent sniffing objects



b) OBAT - Time spent sniffing objects



c) 3CT (Phase 2) - Time spent in the chambers



c) 3CT (Phase 3) - Time spent in the chambers

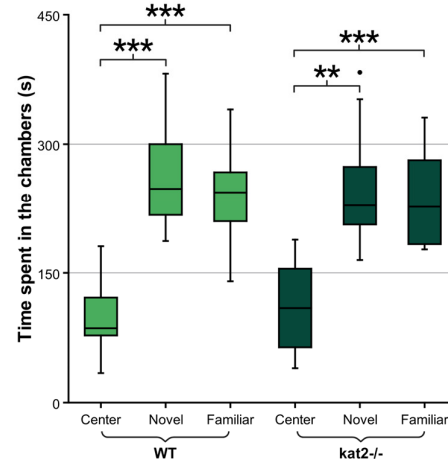


Figure 2. Behavioral assessment of wild-type (WT) and *kat2*^{-/-} mice in object recognition and social interaction paradigms. a) Time spent sniffing familiar vs. novel objects in the novel object recognition test (NORT). During the NORT, both WT and *kat2*^{-/-} animals spent significantly more time exploring the novel object. b) Time spent sniffing familiar vs. novel objects in the object-based attention test (OBAT). In the OBAT, the mutant strain spent more time with the novel object. c) Time spent in the center, social, and non-social chambers during the three-chamber test (3CT, Phase 2). Both WT and *kat2*^{-/-} mice spent more time in the side chambers than in the center chamber. d) Time spent in the center, novel animal's, and familiar animal's chambers during the 3CT (Phase 3). Both WT and *kat2*^{-/-} mice spent more time in the side chambers than in the center chamber. Wild-type mice (light green); *kat2*^{-/-} mice (dark green). Data are presented as mean $\pm$ SD. •, outlier. *, p < 0.05; **, p < 0.01; ***, p < 0.001. The figure was created with LabPlot 2.9.0 (KDE, Berlin, Germany) and BioRender.com. 3CT, three-chamber test; *kat2*^{-/-}, kynurenine aminotransferase II knockout mice; NORT, novel object recognition test; OBAT, object-based attention test; WT, wild-type mice.

3.1.2. Object-Based Attention Test (OBAT)

In the OBAT, no statistically significant differences were detected between the strains in terms of overall object interaction time. Nonetheless, animals from the *kat2*^{-/-} strain exhibited a marked preference for the novel object, spending significantly more time engaging with it compared to the familiar object during testing phase (Figure 2, Table 1, Table 2).

3.1.3. Three Chamber Test (3CT)

During the 3CT, both examined strains spent significantly less time in the lateral chambers compared to the central starting chamber in both the second and third phases of the test. Apart from this difference, no other significant variations were observed (Figure 2, Table 1, Table 2).

3.1.4. Other Behavioral Tests

No significant differences were observed between the strains or within strains in the Y-maze, MBT, and rotarod tests (Figure 2, Table 1).

3.2. Ultra-High-Performance Liquid Chromatography with Tandem Mass Spectrometry (UHPLC-MS/MS)

Several differences were observed between the WT and mutant strains in the various brain regions examined during the chemical analytical measurements (Figure 3, Figure 4, Figure 5, Table 3). The most prominent difference was observed in the level of 3-HK, which was uniformly and significantly increased in all examined brain regions of the *kat2*^{-/-} strain compared to the WT. In a similar pattern, xanthurenic acid (XA) levels were reduced across all analyzed regions relative to the WT. In contrast, the concentration of KYNA, whose alteration was most strongly anticipated, exhibited a significant reduction only in the CTX and HIP; however, the level of KYNA is increased in the STR. Interestingly, the level of its downstream metabolite, quinaldic acid (QAA), remained unaltered in these same areas, while a marked decrease was observed in the CRB and STEM.

Table 3. Regional brain concentrations of tryptophan (Trp) and its downstream metabolites in wild-type (WT) and *kat2*^{-/-} mice across striatum, cortex, hippocampus, cerebellum, and brainstem.

Metabolite	Striatum (nM)			Cortex (nM)			Hippocampus (nM)			Cerebellum (nM)			Brainstem (nM)		
	Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value
	WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}	
Trp	25511.111	21988.889	p < 0.098	30780.00	24090.00	p < 0.004	30433.33	25000.00	p < 0.047	29530.00	25410.00	p < 0.092	29210.00	24730.00	p < 0.121
	±	±		0	0		3	0		0	0		0	0	
	5688.243	1940.647		±	±		±	±		±	±		±	±	
KYN	146.444	152.000	p < 0.768	123.880	121.230	p < 0.867	126.822	124.600	p < 0.886	204.000	129.880	p < 0.215	132.130	119.560	p < 0.483
	± 32.423	± 45.031		± 27.763	± 40.829		± 25.699	± 38.033		±	± 56.356		± 37.311	± 41.100	
										173.369					
KYNA	1.757	3.561	p < 0.026	5.428	2.963	p < 0.027	2.683	1.787	p < 0.026	5.347	4.832	p < 0.417	4.315	3.273	p < 0.058
	± 0.623	± 2.124		± 3.032	± 1.108		± 0.885	± 0.647		± 0.925	± 1.728		± 1.303	± 0.974	
				*			*			*			*		
QAA	no data	no data	no data	0.812	0.714	p < 0.349	0.765	0.807	p < 0.671	0.416	0.249	p < 0.040	0.469	0.171	p < 0.012
				± 0.298	± 0.127		± 0.216	± 0.188		± 0.210	± 0.113		± 0.323	± 0.102	
													*		
AA	2.534	2.431	p < 0.825	0.320	0.461	p < 0.036	0.323	0.213	p < 0.050	0.400	0.296	p < 0.192	0.554	0.477	p < 0.523
	± 0.771	± 1.145		± 0.089	± 0.176		± 0.136	± 0.077		± 0.148	± 0.193		± 0.209	± 0.308	
							*								
3-HK	43.078	78.644	p < 0.019	50.230	87.210	p < 0.019	57.667	104.467	p < 0.006	77.460	118.100	p < 0.044	47.070	83.740	p < 0.006
	± 7.418	± 40.163		± 15.967	± 42.381		± 15.585	± 41.026		± 30.378	± 51.104		± 12.161	± 35.505	
				*			*			**			*		
XA	1.906	1.342	p < 0.224	7.316	2.516	p < 0.019	1.698	0.885	p < 0.015	2.402	1.349	p < 0.015	3.945	1.324	p < 0.001
	± 1.161	± 0.662		± 5.644	± 1.631		± 0.800	± 0.406		± 0.838	± 0.903		± 2.037	± 0.854	
							*			*			*		
3-HAA	no data	no data	no data	6.581	10.128	p < 0.021	3.820	3.994	p < 0.863	no data	no data	no data	no data	no data	no data
				± 2.409	± 3.732		± 1.574	± 2.532							
							*								

QA	20.337 ± 11.449	32.537 ± 15.421	p < 0.075	35.650 ± 11.656	32.946 ± 20.266	p < 0.719	18.296 ± 7.872	24.667 ± 11.260	p < 0.183	27.057 ± 21.057	20.015 ± 12.182	p < 0.372	28.390 ± 16.309	30.201 ± 13.870	p < 0.792
PA	223.877 ± 45.544	233.296 ± 67.819	p < 0.734	146.707 ± 43.541	171.643 ± 46.369	p < 0.231	165.885 ± 81.710	146.251 ± 46.419	p < 0.540	213.970 ± 57.567	291.345 ± 135.202	p < 0.152	220.273 ± 63.283	246.122 ± 89.874	p < 0.467
Serotonin pathway															
5-HTP	55.689 ± 11.051	37.989 ± 20.492	p < 0.037 *	80.070 ± 26.609	37.490 ± 15.897	p < 0.001 ***	48.167 ± 15.514	42.011 ± 9.346	p < 0.323	11.708 ± 4.638	6.451 ± 2.871	p < 0.007 **	65.120 ± 19.882	65.200 ± 34.451	p < 0.995
5-HT	2854.444 ± 281.741	3087.778 ± 318.856	p < 0.119	2780.000 ± 364.722	3238.000 ± 304.478	p < 0.007 **	3162.222 ± 368.571	3316.667 ± 482.519	p < 0.457	382.600 ± 182.449	423.500 ± 218.472	p < 0.655	3542.000 ± 426.375	3554.000 ± 334.006	p < 0.945
5-HIAA	2771.111 ± 232.886	2825.556 ± 493.739	p < 0.769	2816.000 ± 511.147	2634.000 ± 333.340	p < 0.358	3984.444 ± 640.666	3618.889 ± 412.566	p < 0.169	952.300 ± 165.692	954.100 ± 216.632	p < 0.984	3998.000 ± 733.664	4076.000 ± 651.804	p < 0.804
Indole-pyruvate pathway															
IAA	263.000 ± 94.166	196.111 ± 59.711	p < 0.091	174.300 ± 46.294	160.100 ± 32.385	p < 0.437	179.000 ± 41.985	113.767 ± 30.709	p < 0.002 **	47.260 ± 26.084	62.300 ± 18.091	p < 0.151	126.790 ± 38.684	106.450 ± 17.163	p < 0.146
ICA	52.522 ± 15.104	67.000 ± 25.999	p < 0.168	53.590 ± 10.768	85.520 ± 23.399	p < 0.001 ***	58.078 ± 12.274	48.344 ± 7.459	p < 0.059	46.850 ± 16.225	70.060 ± 31.928	p < 0.055	57.430 ± 18.870	56.190 ± 21.972	p < 0.894
IPA	no data	no data	no data	no data	no data	no data	no data	no data	no data	14.509 ± 7.547	12.995 ± 6.244	p < 0.631	29.370 ± 14.081	19.479 ± 5.821	p < 0.055
ILA	88.656 ± 49.392	54.078 ± 17.784	p < 0.066	91.820 ± 24.495	74.690 ± 11.764	p < 0.062	122.067 ± 43.164	91.444 ± 23.630	p < 0.080	62.750 ± 18.591	59.500 ± 12.143	p < 0.649	106.910 ± 19.352	68.030 ± 12.774	p < 0.001 ***
INS	136.444 ± 124.642	66.144 ± 22.154	p < 0.115	181.320 ± 108.171	129.140 ± 54.474	p < 0.190	102.700 ± 93.846	48.411 ± 15.970	p < 0.106	114.680 ± 46.885	73.380 ± 32.000	p < 0.034 *	135.570 ± 66.490	86.080 ± 27.670	p < 0.043 *
pCS	22.863 ± 45.643	6.428 ± 3.057	p < 0.297	13.201 ± 15.641	6.452 ± 6.191	p < 0.221	11.421 ± 14.434	2.932 ± 1.870	p < 0.099	28.415 ± 45.171	4.284 ± 2.878	p < 0.109	5.987 ± 5.293	4.280 ± 4.371	p < 0.442
Tyrosine-dopamine pathway															
Tyr	55533.333 ± 25146.620	33347200.000 ± 66.11.354	p < 0.351	74760.00 0 ± 27036.85 6	55710.00 0 ± 9047.216	p < 0.049 *	2 ± 33835.51 3	4 ± 10032.33 7	p < 0.134	0 ± 27354.98 3	57480.00 0 ± 6273.542	p < 0.112	0 ± 25030.02 6	53600.00 0 ± 9267.026	p < 0.110
L-DOPA	no data	no data	no data	130.840 ± 71.182	119.180 ± 91.862	p < 0.755	147.389 ± 60.587	122.400 ± 31.455	p < 0.288	97.580 ± 16.040	103.530 ± 54.909	p < 0.746	144.090 ± 139.318	118.740 ± 19.909	p < 0.576
3OMD	43.067 ± 11.017	48.467 ± 22.082	p < 0.521	42.010 ± 7.379	46.620 ± 10.305	p < 0.265	42.867 ± 13.189	41.678 ± 9.240	p < 0.828	44.220 ± 8.036	38.350 ± 6.488	p < 0.089	36.990 ± 11.834	35.540 ± 8.342	p < 0.755
DA	238805.322 ± 62124.925	3226596.94 ± 7109.386	p < 0.734	11460.00 ± 4415.938	9733.000 ± 1997.838	p < 0.265	327.778 ± 184.660	301.556 ± 119.914	p < 0.726	126.260 ± 129.803	90.500 ± 32.822	p < 0.409	373.800 ± 137.416	320.700 ± 78.006	p < 0.302
3-MT	12277.778 ± 3429.001	15417.778 ± 7109.386	p < 0.250	2637.000 ± 1213.370	3510.000 ± 2184.272	p < 0.284	72.811 ± 27.012	76.333 ± 42.626	p < 0.837	48.360 ± 51.290	39.780 ± 27.131	p < 0.646	108.990 ± 55.943	100.420 ± 45.985	p < 0.713
DOPAC	9731.111 ± 2098.681	8508.889 ± 2173.571	p < 0.243	3231.000 ± 662.528	2604.000 ± 1298.026	p < 0.190	99.844 ± 31.080	119.122 ± 46.575	p < 0.317	118.510 ± 70.272	58.230 ± 22.991	p < 0.019 *	286.600 ± 83.187	269.700 ± 90.096	p < 0.668

	8845.556	9957.778		3219.000	2994.000		563.667	622.000		241.000	235.410		512.900	479.100	
HVA	±	±	p < 0.334	±	±	p < 0.458	±	±	p < 0.642	± 72.399	±	p < 0.895	±	±	p < 0.668
	1621.983	2933.235		465.271	815.383		249.747	272.426			110.270		142.529	199.441	
VMA	no data	no data	no data	6.815 ± 4.531	9.876 ± 7.149	p < 0.268	4.113 ± 2.537	7.131 ± 3.580	p < 0.056	23.772 ± 34.420	34.862 ± 38.254	p < 0.504	7.121 ± 4.828	8.801 ± 6.926	p < 0.537
MHPGS	31.467 ± 15.193	33.022 ± 10.663	p < 0.805	42.420 ± 20.320	47.070 ± 7.532	p < 0.506	49.722 ± 16.118	46.011 ± 9.992	p < 0.565	23.668 ± 27.307	17.066 ± 6.494	p < 0.467	21.700 ± 9.295	22.040 ± 5.288	p < 0.921
BIO	43.611 ± 16.815	55.533 ± 18.984	p < 0.178	22.410 ± 7.193	18.823 ± 10.159	p < 0.374	15.201 ± 11.095	14.789 ± 12.229	p < 0.941	15.690 ± 3.897	9.233 ± 3.056	P < 0.001 ***	62.380 ± 19.428	41.020 ± 9.643	P < 0.006 **
BH2	514.956 ± 164.623	441.378 ± 173.194	p < 0.369	no data	no data	no data	no data	no data	no data	117.947 ± 16.544	76.528 ± 22.014	P < 0.001 ***	222.409 ± 82.207	192.147 ± 59.659	p < 0.359

Mean ± SD. Asterisks indicate significance levels: *, p < 0.05; **, p < 0.01; ***, p < 0.001. 3-HAA, 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; 3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin (5-hydroxytryptamine); 5-HTP, 5-hydroxytryptophan; AA, anthranilic acid; BH2, dihydrobiopterin; BIO, biopterin; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; ICA, indole-3-carboxaldehyde; IAA, indole-3-acetic acid; ILA, indole-3-lactic acid; INS, indoxyl sulfate; IPA, indole-3-propionic acid; KYN, kynurenine; KYNA, kynurenic acid; L-DOPA, levodopa; 3OMD, 3-O-methyldopa; MHPGS, 3-methoxy-4-hydroxyphenylglycol sulfate; PA, picolinic acid; pCS, p-Cresyl sulfate; QA, quinolinic acid; QAA, quinaldic acid; Trp, tryptophan; Tyr, tyrosine; VMA, vanillylmandelic acid; XA, xanthurenic acid.

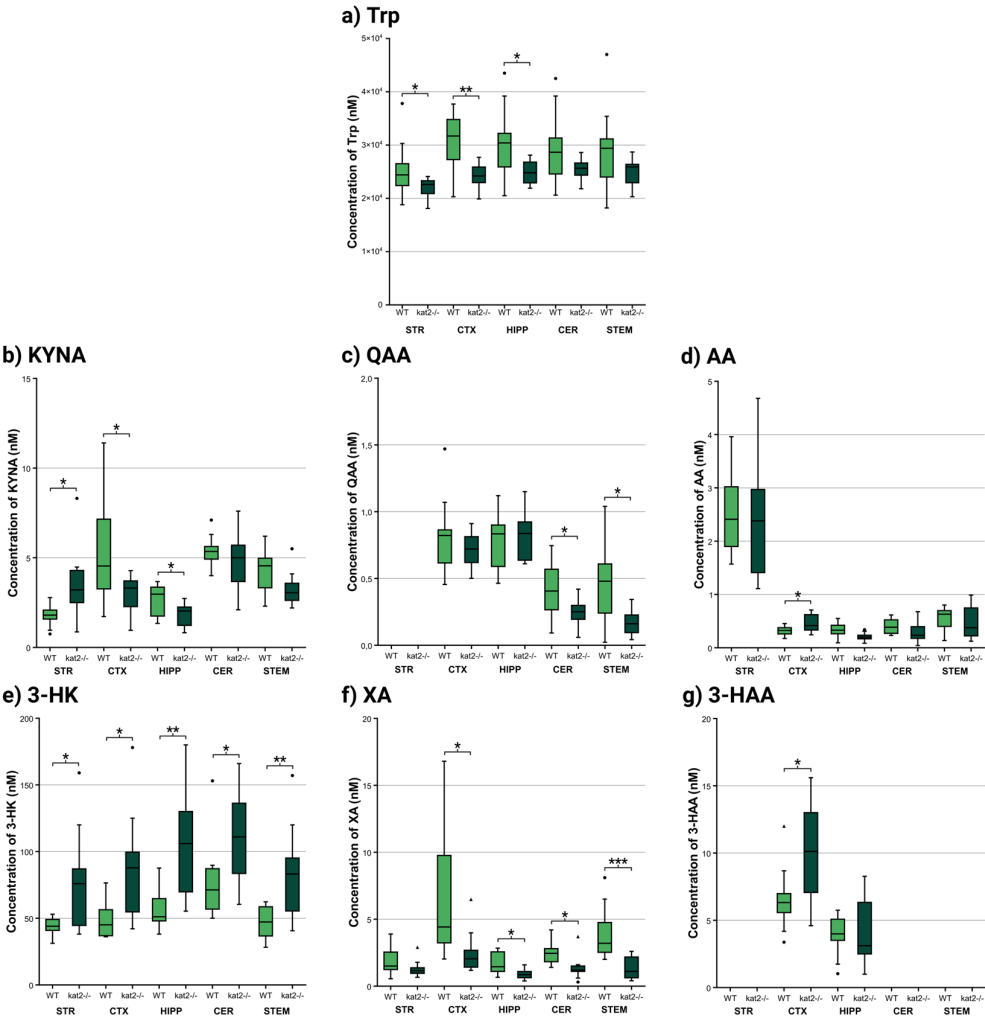


Figure 3. Regional distribution of kynurenine (KYN) pathway metabolites in wild-type (WT) and *kat2*^{-/-} mouse brains. a) Tryptophan (Trp), b) Kynurenic acid (KYNA), c) Quinaldic acid (QAA), d) Anthranilic acid (AA), e) 3-hydroxykynurenine (3-HK), f) Xanthurenic acid (XA), and g) 3-Hydroxyanthranilic acid (3-HAA) concentrations measured in striatum (STR), cortex (CTX), hippocampus (HIPP), cerebellum (CER), and brainstem (STEM). Trp was significantly lower in STR, CTX, and HIPP. While KYNA increased in STR, its concentration lowered in CTX and HIPP. QAA's concentration was significantly lower in CER and STEM. The level of AA was higher in CTX. Concentration of 3-HK increased in every brain region. XA decreased in CTX, HIPP, CER and STEM. The level of 3-HAA increased in CTX. Wild-type (WT, light green) and *kat2*^{-/-} (dark green) groups are shown. Data are expressed as mean $\pm$ SD. •, outlier; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. The figure was created with LabPlot 2.9.0 (KDE, Berlin, Germany) and BioRender.com. 3-HAA, 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; AA, anthranilic acid; CER, cerebellum; CTX, cortex; HIPP, hippocampus; KYNA, kynurenic acid; QAA, quinaldic acid; STEM, brainstem; STR, striatum; Trp, tryptophan; WT, wild-type mice; *kat2*^{-/-}, kynurenine aminotransferase II knockout mice; XA, xanthurenic acid.

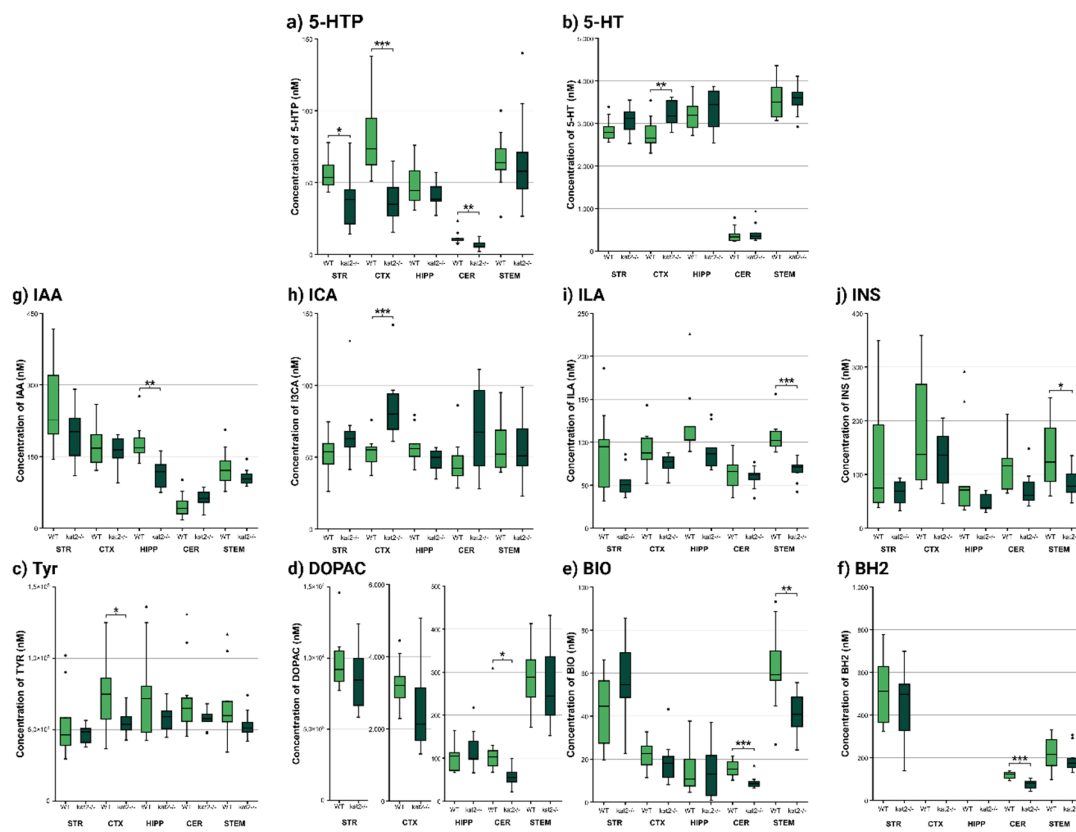


Figure 4. Region-specific alterations in serotonin (5-HT)-, indole-pyruvate-, and tyrosine (Tyr)-dopamine (DA)-derived metabolites in wild-type (WT) and *kat2*^{-/-} mice. Box plots showing concentrations of metabolites across brain regions in wild-type (WT, light green) and *kat2*^{-/-} (dark green) mice. a–b) Serotonin pathway: 5-HTP (5-hydroxytryptophan), 5-HT (5-hydroxytryptamine, serotonin). c–f) Indole-pyruvate pathway: Tyr, DOPAC (3,4-dihydroxyphenylacetic acid), BIO (biopterin), BH2 (dihydrobiopterin). g–j) Tryptophan-derived indole metabolites: IAA (indole-3-acetic acid), ICA (indole-3-carboxaldehyde), ILA (indole-3-lactic acid), INS (indoxyl sulfate). Brain regions: STR, striatum; CTX, cortex; HIPP, hippocampus; CER, cerebellum; STEM, brainstem. 5-HTP concentrations were reduced in the STR, CTX, and CER, whereas 5-HT levels were selectively decreased in the CTX. IAA concentrations were diminished in the HIPP, ILA, and INS within the STEM, while ICA levels were elevated in the CTX. Tyr levels were reduced in the CTX, and decreases in DOPAC, BIO, and BH2 were detected in the CER. Data are shown as mean $\pm$ SD. Symbols: •, outlier; ▲, far out. Significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Figures created with LabPlot 2.9.0 (KDE, Berlin, Germany) and BioRender.com. 5-HT, serotonin (5-hydroxytryptamine); 5-HTP, 5-hydroxytryptophan; BH2, dihydrobiopterin; BIO, biopterin; CER,

cerebellum; CTX, cortex; DOPAC, 3,4-dihydroxyphenylacetic acid; HIPP, hippocampus; ICA, indole-3-carboxaldehyde; IAA, indole-3-acetic acid; ILA, indole-3-lactic acid; INS, indoxyl sulfate; KAT2^{-/-}, kynurenine aminotransferase II knockout mice; STEM, brainstem; STR, striatum; Tyr, tyrosine; WT, wild-type mice.

In addition to the detailed concentration values presented above, a region- and matrix-integrated overview of metabolite alterations is provided (Table 3). Consistent across all examined brain regions, 3-HK was significantly elevated, whereas XA showed a uniform reduction, underscoring a shift toward an oxidative and excitotoxic milieu. KYNA exhibited a divergent pattern, being decreased in the CTX and HIPP yet elevated in the STR, while QAA was selectively reduced in the CER and STEM. Within the serotonergic pathway, 5-hydroxytryptophan (5-HTP) declined in CTX and CER, whereas 5-HT increased in CTX and urine, indicating altered turnover. Further changes included decreased Tyrosine in CTX and HIPP and selective reductions in pterins. Peripheral findings in plasma and urine, previously published, are integrated here for comparison, emphasizing the concordance between central and systemic metabolic rewiring.

Complementing our previous measurements of Trp metabolite levels in plasma and urine, we also measured the concentrations of additional metabolites from the indole-pyruvate and TYR-DA pathways; however, we observed significant changes only in 3-methoxy-4-hydroxyphenylglycol sulphate (MHPGS) levels compared to WT (Table 4, Figure 5).

Table 4. Concentrations of indole-pyruvate and tyrosine-dopamine pathway metabolites in wild-type (WT) and *kat2^{-/-}* mice in plasma and urine.

Metabolite	Plasm (nM)			Urine (nM)		
	Mean ± SD		p-value	Mean ± SD		p-value
	WT	<i>kat2^{-/-}</i>		WT	<i>kat2^{-/-}</i>	
Indole-pyruvate pathway						
ICA	no data	no data	no data	no data	no data	no data
IPA	no data	no data	no data	no data	no data	no data
ILA	no data	no data	no data	no data	no data	no data
pCS	853.520 ± 961.663	1097.193 ± 1196.572	p < 0.622	9683.873 ± 15558.939	7429.639 ± 12598.662	p < 0.726
Tyrosine-dopamine pathway						
Tyr	50824.432 ± 20811.617	35775.857 ± 16975.863	p < 0.093	9411.420 ± 2214.266	8789.288 ± 1547.575	p < 0.476
L-DOPA	36.800 ± 15.606	35.109 ± 13.708	p < 0.800	no data	no data	no data
3OMD	36.340 ± 5.556	31.128 ± 5.595	p < 0.051	41.828 ± 21.255	40.299 ± 17.829	p < 0.864
DA	no data	no data	no data	671.105 ± 320.951	779.887 ± 193.877	p < 0.371
3-MT	2.653 ± 1.315	1.796 ± 0.655	p < 0.082	241.517 ± 93.336	235.939 ± 50.578	p < 0.870
DOPAC	no data	no data	no data	370.196 ± 224.797	301.471 ± 108.291	p < 0.395
HVA	no data	no data	no data	1120.520 ± 890.606	731.657 ± 173.621	p < 0.192
VMA	no data	no data	no data	820.064 ± 567.571	760.459 ± 124.132	p < 0.749
MHPGS	27.657 ± 12.496	15.392 ± 6.886	p < 0.014 *	8533.153 ± 3929.104	14639.178 ± 3364.617	p < 0.002 **
BIO	68.419 ± 23.013	82.535 ± 17.725	p < 0.142	231.328 ± 80.142	221.133 ± 88.018	p < 0.790
BH2	588.898 ± 122.352	577.701 ± 178.965	p < 0.872	4508.851 ± 2655.882	4488.304 ± 2298.353	p < 0.985

Mean ± SD. Asterisks indicate significance levels: *, $p < 0.05$; **, $p < 0.01$. 3OMD, 3-O-methyldopa; 3-MT, 3-methoxytyramine; BH2, dihydroxybiopterin; BIO, biopterin; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; ICA, indole-3-carboxaldehyde; ILA, indole-3-lactic acid; IPA, 3-indolepropionic acid; *kat2*^{-/-}, kynurenine aminotransferase II knockout mice; L-DOPA, levodopa; MHPGS, 3-methoxy-4-hydroxyphenylglycol sulphate; pCS, para-Cresol sulphate; SD, standard deviance; Tyr, tyrosine; VMA, vanillylmandelic acid; WT, wild-type mice.

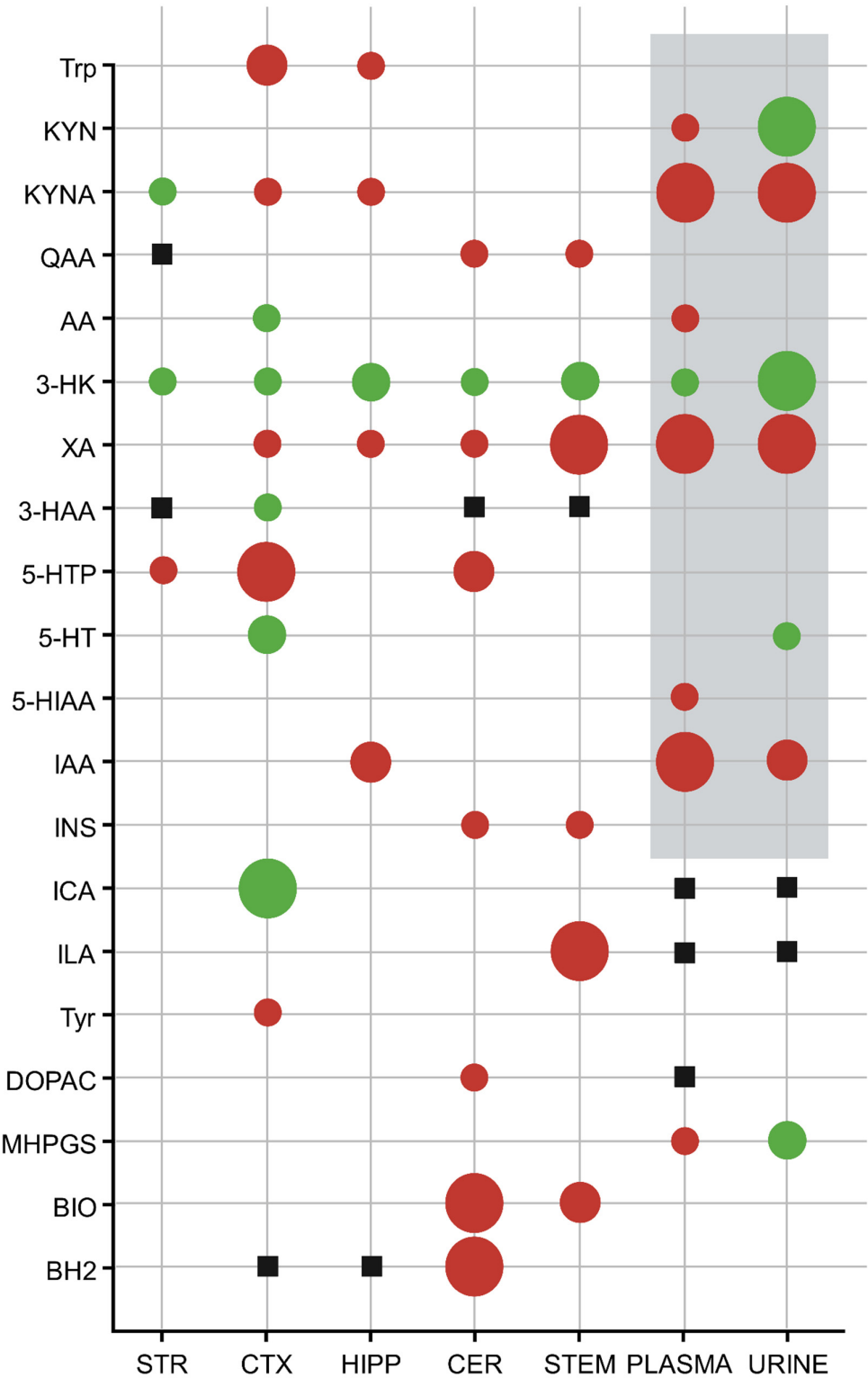


Figure 5. Overview of region- and matrix-specific alterations in tryptophan-derived metabolites in 8-week-old male *kat2^{-/-}* mice compared to wild-type (WT) controls in ultra-high-plasticity liquid chromatography with tandem mass spectrometry. This figure summarizes significant alterations in tryptophan–kynurenine, serotonin, indole–pyruvate, and tyrosine–dopamine pathway metabolites across distinct brain regions (striatum, cortex, hippocampus, cerebellum, brainstem), plasma and urine in *kat2^{-/-}* mice compared to the WT. Results highlight the region- and pathway-selective metabolic rewiring induced by *kat2* deletion, particularly the consistent increase of 3-hydroxykynurenine and decrease of xanthurenic acid, alongside mixed kynurenine acid responses and downstream shifts in serotonin, indole, and catecholamine derivatives. We marked significant changes with circles. Red circles mean statistically significant decrease, and green shows significant increase in the concentration compared to the WT mice. The increasing size of the circles indicates higher levels of significance (small circle: *, *p* < 0.05; medium circle: **, *p* < 0.01; large circle: ***, *p* < 0.001). Grey rectangle background: previously published results. Black square: no data. 3-HAA, 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HT, serotonin (5-hydroxytryptamine); 5-HTP, 5-hydroxytryptophan; AA, anthranilic acid; BH2, dihydroxybiopterin; BIO, biopterin; CER, cerebellum; CTX, cortex; DOPAC, 3,4-dihydroxyphenylacetic acid; HIP, hippocampus; IAA, indole acetic acid; ICA, indole-3-carboxaldehyde; ILA, indole-3-lactic acid; INS, indoxyl sulphate; KYN, kynurenine; KYNA, kynurenic acid; MHPGS, 3-methoxy-4-hydroxyphenylglycol sulphate; QAA, quinaldic acid; Trp, tryptophan; Tyr, tyrosine; STEM, brainstem; STR, striatum; XA, xanthurenic acid.

3.3. Enzyme Activities

Enzyme activity ratios revealed pronounced remodeling of tryptophan metabolic fluxes in *kat2^{-/-}* mice (Figure 6, Table 5). As expected, KAT activity (KYN/Trp) displayed a selective reduction in the STR, consistent with the genetic deletion of KAT II. Conversely, KMO activity (3-HK/KYN) was markedly elevated across STR, CTX, HIP, CER, and STEM, indicating enhanced pro-oxidant pressure through 3-HK production. KYNU activity (3-HAA/3-HK) showed a modest yet significant increase in the HIP, while KAT III activity (XA/3-HK) was consistently reduced in CTX, HIP, CER, and STEM, reinforcing the loss of protective XA formation. Within the serotonergic arm, tryptophan hydroxylase (TPH) activity (5-HTP/Trp) was reduced in CTX and CER, whereas aromatic L-amino acid decarboxylase (AADC) activity (5-HT/5-HTP) was elevated in STR, CTX, and HIP, suggesting compensatory 5-HT turnover. MAO/ALDH activity (5-HIAA/5-HT) was decreased only in CTX, while TMO activity (IAA/Trp) was reduced in CTX, HIP, and CER. Collectively, these shifts highlight a pathway-specific reorganization, with KMO dominance, curtailed XA buffering, and altered 5-HT–indole dynamics shaping the *kat2^{-/-}* metabolic phenotype.

Table 5. Ratios of tryptophan (Trp)-derived metabolites to their precursors and associated enzyme activities across brain regions in wild-type (WT) and *kat2^{-/-}* mice (mean ± SD, *p*-values). Activities were estimated using product-to-substrate ratios for key enzymatic steps: TDO/IDOs (KYN/Trp), KATs (KYNA/KYN), KMO (3-HK/KYN), KYNU (AA/KYN; 3-HAA/3-HK), KAT III (XA/3-HK), 3-HAO (QA/3-HAA; PA/3-HAA), TPHs (5-HTP/Trp), AADC (5-HT/5-HTP), MAOs + ALDH (5-HIAA/5-HT), TMO (IAA/Trp), and TNA (INS/Trp).

Enzyme	Product/Substrate	Striatum			Cortex			Hippocampus			Cerebellum			Brainstem		
		Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value
		WT	<i>kat2^{-/-}</i>		WT	<i>kat2^{-/-}</i>		WT	<i>kat2^{-/-}</i>		WT	<i>kat2^{-/-}</i>		WT	<i>kat2^{-/-}</i>	
TDO/IDOs	KYN/Trp	0.006	0.007	p < 0.188	0.004	0.005	p < 0.212	0.004	0.005	p < 0.245	0.006	0.005	p < 0.349	0.005	0.005	P < 0.907
		± 0.001	± 0.002		± 0.001	± 0.001		± 0.001	± 0.001		± 0.004	± 0.002		± 0.001	± 0.002	
KATs	KYNA/KYN	0.012	0.026	p < 0.035 *	0.045	0.026	p < 0.063	0.022	0.015	p < 0.099	0.036	0.041	p < 0.487	0.034	0.032	P < 0.696
		± 0.005	± 0.017		± 0.027	± 0.012		± 0.010	± 0.007		± 0.015	± 0.019		± 0.011	± 0.017	
KMO	3-HK/KYN	0.305	0.510	p < 0.002 **	0.413	0.714	p < 0.001 **	0.463	0.844	p < 0.001 ***	0.458	0.957	p < 0.001 ***	0.365	0.709	P < 0.001 ***
		± 0.075	± 0.216		± 0.131	± 0.262		± 0.121	± 0.264		± 0.139	± 0.365		± 0.087	± 0.230	

KYNU	AA/KYN	0.018 ± 0.006	0.018 ±0.009	p< 0.951	0.003 ± 0.001	0.004 ±0.003	p< 0.208	0.003 ± 0.001	0.002 ± 0.001	p< 0.301	0.003 ± 0.001	0.003 ± 0.002	p< 0.913	0.004 ± 0.002	0.005 ± 0.004	P< 0.651
KYNU	3-HAA/3-HK	no data	no data	no data	0.135 ± 0.040	0.125 ±0.034	p< 0.540	0.072 ± 0.036	0.038 ± 0.020	p< 0.026 *	no data	no data	no data	no data	no data	no data
KAT III	XA/3-HK	0.045 ± 0.027	0.022 ±0.018	p< 0.052	0.151 ± 0.106	0.035 ±0.027	p< 0.001 **	0.031 ± 0.017	0.011 ± 0.009	p< 0.007 **	0.034 ± 0.015	0.014 ± 0.015	p< 0.001 **	0.088 ± 0.054	0.020 ± 0.017	P< 0.001 ***
3-HAO	QA/3-HAA	no data	no data	no data	5.910 ± 2.878	3.693 ±2.022	p< 0.112	6.125 ± 4.070	10.220 ± 9.885	p< 0.508	no data	no data	no data	no data	no data	no data
3-HAO + ACMSD	PA/3-HAA	no data	no data	no data	23.688 ± 7.982	20.024 ± 13.316	p< 0.082	66.477 ± 82.924	51.852 ± 43.947	p< 0.895	no data	no data	no data	no data	no data	no data
TPHs	5-HTP/Trp	0.002 ± 0.000	0.002 ±0.001	p< 0.313	0.003 ± 0.001	0.002 ±0.001	p< 0.005 **	0.002 ± 0.000	0.002 ± 0.000	p< 0.331	0.000 ± 0.000	0.000 ± 0.000	p< 0.003 **	0.002 ± 0.001	0.003 ± 0.001	P< 1.000
AADC	5-HT/5-HTP	53.246 ± 12.810	110.941 ± 73.047	p< *	37.833 ± 12.360	102.426 ± 43.532	p< **	72.470 ± 26.437	82.603 ± 72.339	p< 0.393	34.597 ± 17.138	774.676 ± 41.567	p< 0.004 **	63.557 ± 38.662	768.894 ± 237.313	P< 0.496
MAOs + ALDH	5-HIAA/5-HT	0.982 ± 0.148	0.922 ±0.180	p< 0.453	1.036 ± 0.280	0.822 ±0.141	p< 0.044 *	1.290 ± 0.327	1.126 ± 0.285	p< 0.274	2.905 ± 1.239	2.539 ± 0.796	p< 0.442	1.150 ± 0.271	1.160 ± 0.232	P< 0.926
TMO (TrD, ArAT)	IAA/Trp	0.010 ± 0.003	0.009 ±0.002	p< 0.212	0.006 ± 0.002	0.007 ±0.001	p< 0.054	0.006 ± 0.001	0.005 ± 0.001	p< 0.021 *	0.002 ± 0.001	0.002 ± 0.001	p< 0.026 *	0.004 ± 0.001	0.004 ± 0.001	P< 0.597
TNA	INS/Trp	0.005 ± 0.003	0.003 ±0.001	p< 0.554	0.006 ± 0.003	0.005 ±0.002	p< 0.624	0.003 ± 0.002	0.002 ± 0.001	p< 0.161	0.004 ± 0.002	0.003 ± 0.001	p< 0.170	0.005 ± 0.003	0.003 ± 0.001	P< 0.147

Data are expressed as mean ±SD. Significance: *, p<0.05; **, p<0.01; ***, p<0.001. 3-HAA, 3-hydroxyanthranilic acid; 3-HAO, 3-hydroxyanthranilate oxidase; 3-HK, 3-hydroxykynurenine; AA, anthranilic acid; AADC, aromatic L-amino acid decarboxylase; ACMSD, aminocarboxymuconate-semialdehyde decarboxylase; ALDH, aldehyde dehydrogenase; CER, cerebellum; CTX, cortex; HIP, hippocampus; IAA, indole-3-acetic acid; IDOs, indoleamine 2,3-dioxygenases; INS, indoxyl sulfate; *kat2^{-/-}*, kynurenine aminotransferase II knockout mice; KATs, kynurenine aminotransferases; KMO, kynurenine 3-monooxygenase; KYNA, kynurenic acid; KYNU, kynureninase; MAOs, monoamine oxidases; PA, picolinic acid; QA, quinolinic acid; QAA, quinolinic acid analog; STEM, brainstem; STR, striatum; TDO, tryptophan 2,3-dioxygenase; TMO, tryptophan monooxygenase; TNA, tryptophan N-acetyltransferase; TrD, tryptophan deaminase; Trp, tryptophan; Tyr, tyrosine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin (5-hydroxytryptamine); 5-HTP, 5-hydroxytryptophan; WT, wild-type mice; XA, xanthurenic acid.

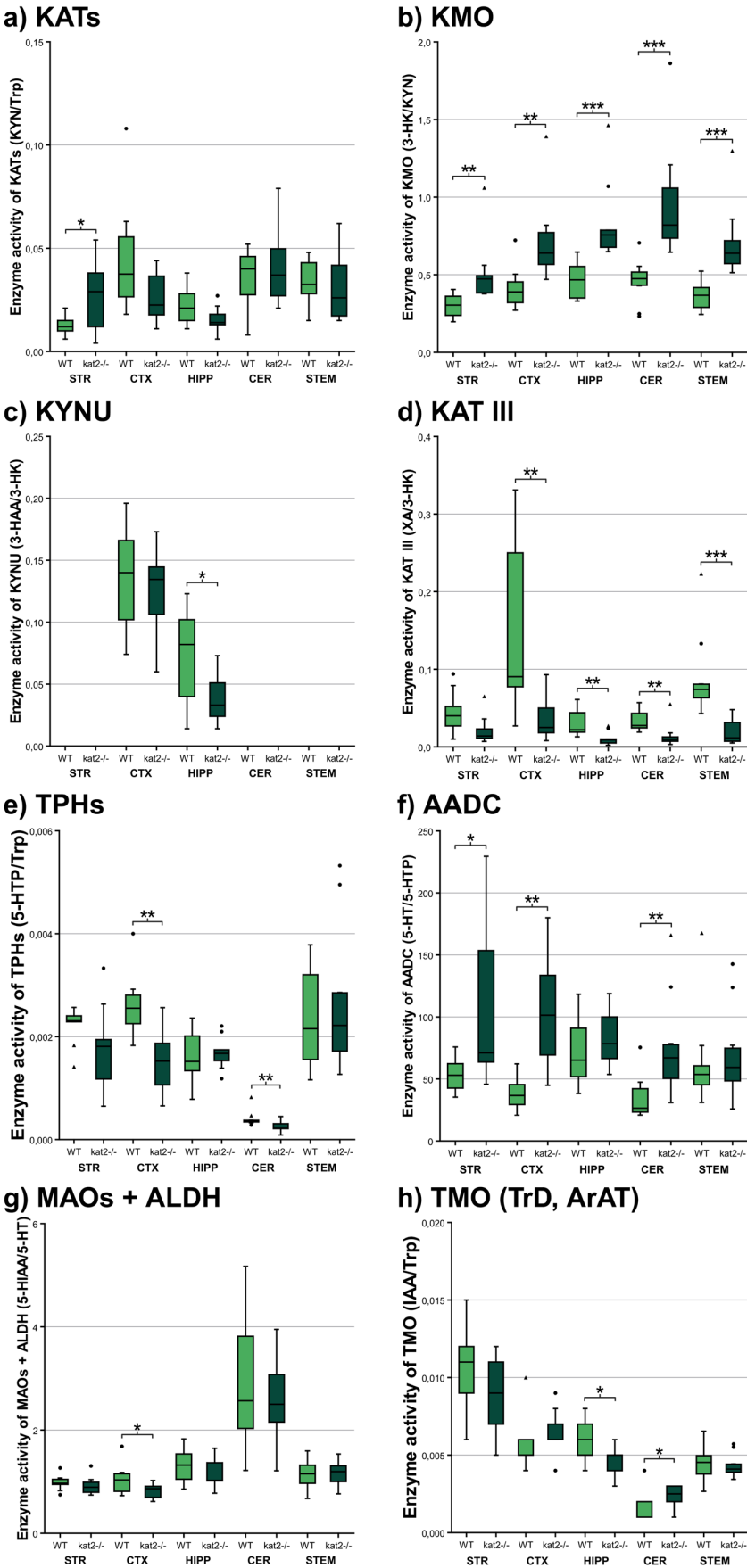


Figure 6. Regional enzyme activity alterations in tryptophan- and serotonin (5-HT)-associated pathways in WT and *kat2*^{-/-} mice. Box plots showing enzyme activities across striatum (STR), cortex (CTX), hippocampus (HIPP), cerebellum (CER), and brainstem (STEM) in wild-type (WT, light green) and *kat2*^{-/-} (dark green) mice. Activities were calculated as product-to-substrate ratios for: a) KATs (KYNA/KYN), b) KMO (3-HK/KYN), c) KYNU (3-HAA/3-HK), d) KAT III (XA/3-HK), e) TPHs (5-HTP/Trp), f) AADC (5-HT/5-HTP), g) MAOs + ALDH (5-HIAA/5-HT), h) TMO [TrD, ArAT] (IAA/Trp). Enzyme activity of KATs decreased in STR. KMO's activity significantly increased in every brain region. KYNU's activity decreased in HIPP. KAT III enzyme's activity decreased in CTX, HIPP, CER, and STEM. Activity of TPH enzymes decreased in CTX and CER. AADC's activity decreased in STR, CTX, and CER. MAOs + ALDH decreased in CTX. TMO's activity decreased in HIPP and CER. Data are shown as mean ± SD. Symbols: ●, outlier; ▲, far out. Statistical significance: *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001. Figures were created with LabPlot 2.9.0 (KDE, Berlin, Germany) and BioRender.com. 3-HAA, 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin (5-hydroxytryptamine); 5-HTP, 5-hydroxytryptophan; AADC, aromatic L-amino acid decarboxylase; ALDH, aldehyde dehydrogenase; ArAT, aromatic amino acid aminotransferase; CER, cerebellum; CTX, cortex; HIPP, hippocampus; IAA, indole-3-acetic acid; KAT III, kynurenine aminotransferase III; KATs, kynurenine aminotransferases; KAT2^{-/-}, kynurenine aminotransferase II knockout; KMO, kynurenine-3-monooxygenase; KYN, kynurenine; KYNU, kynureninase; MAOs, monoamine oxidases; STEM, brainstem; STR, striatum; TMO, tryptophan-2-monooxygenase; TPHs, tryptophan hydroxylases; TrD, tryptophan decarboxylase; Trp, tryptophan; WT, wild-type mice; XA, xanthurenic acid.

3.4. Oxidative Stress and Excitotoxicity Indices

To further evaluate the balance between oxidative pressure and excitotoxic potential, we calculated composite indices from key KYN metabolites (Figure 7, Table 6). The oxidative stress index, defined as 3-HK/(KYNA+AA+XA), was significantly elevated in multiple brain regions of *kat2*^{-/-} mice. Compared to wild-type controls, the CTX, HIPP, CER, and STEM all exhibited marked increases, with the HIPP and STEM showing the most robust elevations. This pattern reflects the combined impact of increased 3-HK and reduced antioxidant metabolites, underscoring a shift toward pro-oxidant load. In contrast, the excitotoxicity index, measured as QA/KYNA, showed a more selective profile. While values remained unchanged in STR, CTX, and CER, a significant increase emerged in the HIPP, where diminished KYNA coincided with elevated QA. A similar trend, though nonsignificant, was observed in the STEM. Taken together, these data indicate that KAT II deficiency imposes a dual burden of oxidative stress and region-specific excitotoxic vulnerability, with the HIPP emerging as a particularly sensitive locus of metabolic imbalance.

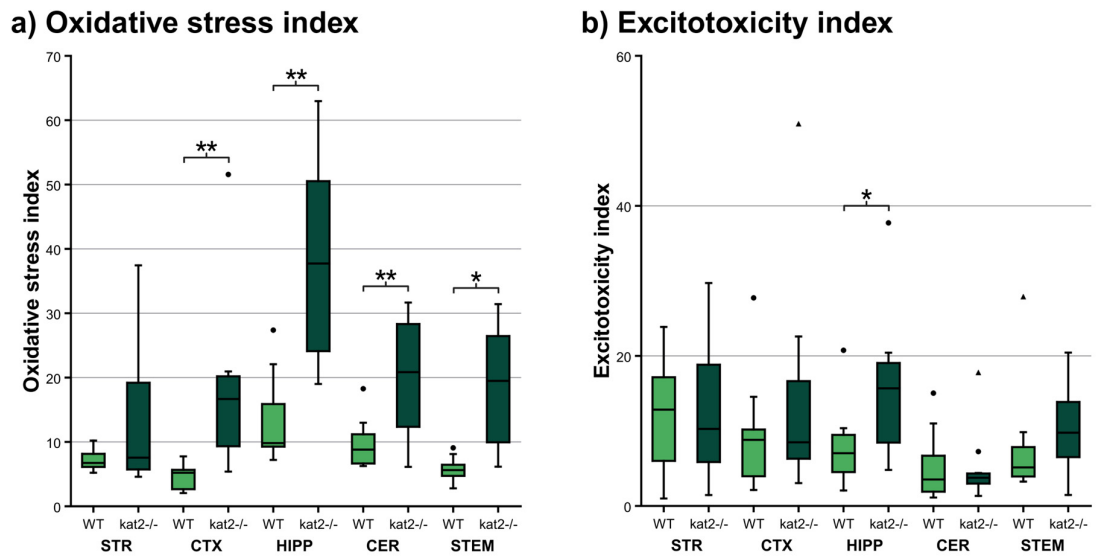


Figure 7. Regional indices of oxidative stress and excitotoxicity in wild-type (WT) and *kat2*^{-/-} mice. Box plots showing oxidative stress and excitotoxicity indices across striatum (STR), cortex (CTX), hippocampus (HIP), cerebellum (CER), and brainstem (STEM) in WT (light green) and *kat2*^{-/-} (dark green) mice. a) Oxidative stress index, calculated as the ratio 3-HK/(KYNA+AA+XA), reflecting the balance between pro-oxidant and antioxidant metabolites. The oxidative stress index significantly increased in CTX, HIP, CER and STEM. b) Excitotoxicity index, calculated as the ratio QA/KYNA, representing the N-methyl-D-aspartate (NMDA) receptor agonist-to-antagonist balance. The excitotoxicity index increased in HIP. Data are shown as mean ± SD. Symbols: •, outlier; ▲, far out. Statistical significance: *, p < 0.05; **, p < 0.01. Figures generated with LabPlot 2.9.0 (KDE, Berlin, Germany) and BioRender.com. 3-HK, 3-hydroxykynurenine; AA, anthranilic acid; CER, cerebellum; CTX, cortex; HIP, hippocampus; *kat2*^{-/-}, kynurenine aminotransferase II knockout mice; KYNA, kynurenic acid; QA, quinolinic acid; STEM, brainstem; STR, striatum; WT, wild-type mice; XA, xanthurenic acid.

Table 6. Ratios of oxidant/antioxidant and N-methyl-D-aspartate (NMDA) agonist/antagonist metabolites across brain regions in wild-type (WT) and *kat2*^{-/-} mice (mean ± SD, p-values). Regional indices of oxidative stress and excitotoxicity in WT and *kat2*^{-/-} mice. Oxidative stress was quantified using the ratio 3-HK/(KYNA+AA+XA), reflecting the balance between pro-oxidant and antioxidant metabolites, while excitotoxic potential was assessed by the ratio QA/KYNA, indicating NMDA receptor agonist/antagonist balance. Measurements were performed across striatum, cortex, hippocampus, cerebellum, and brainstem. Data are presented as mean ± SD.

Oxidant/antioxidant metabolites	Striatum			Cortex			Hippocampus			Cerebellum			Brainstem		
	Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value
	WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}	
3-HK/(KYNA+AA+XA)	6.951	10.723	p < 0.627	4.700	17.670	p < 0.001 **	13.509	37.148	p < 0.002 **	9.667	20.311	p < 0.006 **	5.740	18.705	p < 0.002 **
	±	±		±	±		±	±		±	±		±	±	
	2.904	10.215		2.112	13.315		6.992	15.859		3.839	9.275		1.892	9.300	
NMDA agonist/antagonist metabolites	Striatum			Cortex			Hippocampus			Cerebellum			Brainstem		
	Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value	Mean ± SD		p-value
	WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}		WT	<i>kat2</i> ^{-/-}	
QA/KYNA	11.575	9.138	p < 0.923	9.263	14.235	p < 0.597	7.957	16.120	p < 0.046 *	5.306	4.998	p < 0.880	7.724	10.224	p < 0.096
	±	±		±	±		±	±		±	±		±	±	
	18.379	7.260		7.574	14.254		5.478	9.907		4.601	4.789		7.414	5.544	

Data are expressed as mean ± SD. Significance: *, p < 0.05; **, p < 0.01; ***, p < 0.001. 3-HK, 3-hydroxykynurenine; AA, anthranilic acid; *kat2*^{-/-}, kynurenine aminotransferase II knockout mice; KYNA, kynurenic acid; NMDA, N-methyl-D-aspartate; QA, quinolinic acid; WT, wild-type mice; XA, xanthurenic acid.

Results begin with region-resolved metabolomics across STR, CTX, HIP, CER, and STEM, followed by enzyme-ratio proxies, cofactor mapping, and alignment with behavioral outcomes. A convergent central signature emerges: 3-HK increases and XA decreases pan-regionally, KYNA reduction localizes to CTX and HIP, KMO activity indices rise while KAT III flux falls, and oxidative-stress measures increase broadly, with a hippocampal-specific rise in the excitotoxicity index. Cofactor analyses identify BIO depletion in CER and STEM and BH2 loss in CER. Gut-brain and serotonergic markers also shift, with hippocampal IAA reduction, cortical and cerebellar ICA elevation, and reduced cortical 5-HT turnover, yet cognition, sociability, and coordination remain intact. Consistent with region-specific rewiring rather than a uniform *kat2* effect, we note a paradoxical pattern: striatal KYNA elevation likely reflects compensatory KAT isoforms or astrocyte-mediated KYN shunting; forebrain bias toward AA aligns with oxidative-stress-driven KMO→KYNU flux that diverts 3-HK from KYNA. In sum, findings delineate a KMO-tilted, pterin-constrained, indole-modulated milieu that selectively burdens affective and motor subdomains while sparing core cognitive and social functions.

4. Discussion

The metabolism of Trp has emerged as a central hub in the neurobiology of cognition, mood, and social behavior, with imbalances along its KYN, 5-HT, indole, and DA branches increasingly linked to neuropsychiatric and neurodegenerative disorders [15,16,22,28,39]. Within this network, KAT II has been considered the dominant enzymatic source of KYNA, a metabolite long viewed as neuroprotective through NMDA, α 7-nAChR, AMPA, and kainate antagonism [20,67,125–130]. Yet, earlier reports of global KAT II inhibition or genetic deletion left unresolved how local shifts in KYNA and related metabolites shape functional brain states [20,28,67,126,129,131]. By combining region-resolved metabolomics with behavioral phenotyping, our study addresses this gap, asking whether neurochemical disequilibria in KAT II deficiency necessarily translate into overt cognitive, motor, or social dysfunction [20,22,67,132,133].

The metabolic profile emerging from *kat2*^{-/-} brains reveals a striking shift toward a pro-oxidant milieu, characterized by pan-regional accumulation of 3-HK and consistent loss of XA, with an additional region-selective reduction of KYNA in CTX and HIP. Such changes converge on a biochemical signature that indicates heightened KMO activity alongside impaired KAT II flux, effectively tilting the KYN pathway toward neurotoxic branch products [35,67,134]. The imbalance between reduced antioxidant buffering (XA, KYNA) and sustained excitatory drive amplifies oxidative-stress indices across regions, with the HIP exhibiting a peak excitotoxicity signal. This constellation suggests that, although behavioral performance remained intact, the HIP in particular resides in a precarious metabolic state, vulnerable to secondary insults [135]. Thus, the observed disequilibrium highlights a latent central risk architecture, in which KAT II deficiency may predispose selective circuits to degeneration under stress or aging.

The selective decline of KYNA in HIP and CTX is particularly consequential, as these structures form the backbone of glutamatergic integration underlying memory, attentional control, and affective regulation [126,136–140]. Lower KYNA levels in these regions imply diminished tonic antagonism at NMDA and α 7-nicotinic receptors, a state that can facilitate plasticity yet simultaneously heighten vulnerability to excitotoxic cascades. Such changes resonate with the well-documented link between KYN pathway imbalance and affective or cognitive disturbances. In contrast, the cerebellar and STEM pattern, where QAA and related metabolites decline, speaks to circuits subserving motor coordination and arousal [126,136,137,141]. Here, altered metabolic buffering could subtly recalibrate sensorimotor integration and vigilance states, aligning with CER–STEM contributions to motor timing and autonomic tone. Together, these region-specific shifts underscore circuit-level rebalancing rather than global disruption.

The consistent elevation of 3-HK across regions, paralleled by a decline in XA, underscores a shift toward a redox-imbalanced milieu that favors oxidative stress [142–144]. This pro-oxidant tilt is further amplified when considered in the context of derived indices, where the 3-HK-to-antioxidant ratio signals a vulnerability state rather than an immediate injury [142–144]. In parallel, the excitotoxicity index, weighted by QA/KYNA balance, delineates selective windows in which NMDA drive may outweigh intrinsic antagonism [142,143,145]. These biochemical loads, however, need not manifest uniformly as behavioral deficits at baseline. Instead, they may represent latent liabilities, poised to surface under developmental, aging, or environmental stressors, thereby marking a hidden susceptibility rather than an overt phenotype [15,143,144].

Region-specific perturbations in the pterin pool highlight a subtle but consequential layer of metabolic vulnerability [146–148]. In *kat2*^{-/-} hindbrain, the concurrent depletion of BIO and loss of BH₂ suggest an erosion of the redox-cycling capacity that normally safeguards BH₄ availability [146–148]. Because BH₄ is indispensable for tyrosine hydroxylase activity, even modest shifts in this cofactor equilibrium may attenuate DA biosynthesis and, secondarily, compromise broader monoaminergic tone [146–149]. These alterations could reverberate into nitric oxide biology, given that endothelial and neuronal nitric oxide synthase (NOS) also require BH₄, thereby coupling monoamine insufficiency to redox imbalance and impaired vasomodulation [146–148].

The neurochemical profile in *kat2*^{-/-} mice suggests that reduced serotonergic turnover in the CTX converges with decreased hippocampal IAA and concomitant elevations of ICA in cortical and cerebellar regions to shape circuit-level excitability [150–152]. Lower 5-HT turnover may weaken cortical inhibitory tone, thereby amplifying the impact of indole-derived signaling [151,152]. The reduction of IAA, a ligand with protective barrier and anti-inflammatory properties, contrasts with the elevation of ICA, a potent AhR agonist capable of reprogramming microglial states [150–152]. Through this shift, cortical and cerebellar microglia may adopt transcriptional phenotypes that subtly recalibrate glutamatergic drive and synaptic responsiveness [150–152]. Such AhR-mediated modulation, in concert with altered serotonergic dynamics, delineates a mechanism by which KAT II deficiency reshapes microcircuit stability without overtly impairing cognitive or social behaviors.

The parallel remodeling of Trp metabolism across central and peripheral compartments suggests a degree of concordance that greatly enhances translational traction [15,153,154]. When brain signatures mirror those detected in plasma or urine—such as the uniform elevation of 3-HK and the consistent reduction in XA—biomarker feasibility is strengthened because measurements in accessible fluids reliably report on neurochemical states [15,153,154]. This concordance further supports longitudinal monitoring, since repeated peripheral sampling can index dynamic shifts in pathway fluxes without invasive procedures [15,153,154]. Importantly, the pattern of a KMO-tilted, pterin-constrained phenotype provides a stratification handle: individuals or models exhibiting this profile may constitute a distinct subgroup marked by heightened oxidative burden and diminished neuroprotection [15,131,153]. Thus, the central–peripheral alignment not only validates observed signatures but also operationalizes them for precision tracking and targeted intervention.

Despite a pervasive biochemical tilt toward oxidative stress and excitotoxic vulnerability, baseline cognition and sociability remain largely preserved in *kat2*^{-/-} mice. This dissociation likely reflects both redundancy within cognitive and social circuits and compensatory plasticity that stabilizes performance until a higher stress threshold is breached [67,155,156]. Cortico-hippocampal KYNA loss may sensitize affective and attentional pathways, but parallel buffering via dopaminergic and indole-linked mechanisms appears sufficient to maintain recognition memory and sociability in standard tasks. In contrast, motor and affective domains, subserved by CER–STEM loops and stress-responsive hippocampal circuits, manifest early pressure, consistent with lower redundancy and higher task sensitivity. These findings underscore that metabolic disequilibria need not uniformly generalize to behavior and that endpoint detectability hinges on domain-specific thresholds. For experimental design, this argues against relying solely on cognition- or sociability-based readouts and instead favors composite panels that capture latent affective or motor liabilities, especially when probing therapeutic interventions or stress challenges.

The paradoxical distribution of metabolites across brain regions points to a region-specific metabolic rewiring rather than a uniform effect of *kat2* loss [125,157]. Striatal KYNA increased, while it declined in CTX and HIPPO and remained unchanged in CER and STEM. This striatal KYNA elevation in the absence of *kat2* likely arises from alternative KAT isoform activity or astrocyte-mediated KYN shunting, consistent with the region's dense dopaminergic and glial milieu [158]. In contrast, plasma and urine showed marked KYNA reductions, underscoring that regional enzymatic compensation and astrocytic buffering, rather than systemic availability, govern KYN metabolism within the brain.

Serotonergic metabolism revealed equally complex shifts. 5-HTP fell consistently in STR, CTX, and CER, suggesting precursor depletion, yet cortical 5-HT paradoxically rose. This contrast hints at selective upregulation of decarboxylase activity or altered transporter dynamics in cortical circuits. Importantly, urine samples captured a 5-HT increase, while plasma remained unaltered—underscoring a clear brain–periphery mismatch. Similarly, Trp declined in CTX and HIPPO but stayed unchanged peripherally, whereas KYN itself was stable in brain yet shifted downward in plasma and upward in urine.

Other metabolites highlighted both paradoxical and stable nodes. 3-HK rose broadly across central and peripheral compartments, but its downstream metabolite XA decreased everywhere,

revealing a systemic enzymatic bottleneck. AA, however, increased in CTX but decreased in HIPPI and STEM, with no peripheral reflection. Several intermediates, including Tyr and 3-HAA, remained stable in most regions, pointing to strong compensatory buffering. Taken together, these findings illustrate that *kat2* deletion drives a patchwork of paradoxical imbalances, where regional demands, oxidative stress, and glial density dictate divergent metabolic trajectories, while peripheral readouts capture only a partial, homogenized snapshot of these changes.

Furthermore, the single-gene *kat2* knockout, which markedly reduces KYNA concentrations, exerts consequences that extend far beyond the KYN branch alone. The disruption alters the enzymatic dynamics and metabolite distribution of the Trp–KYN axis, but also propagates into parallel domains such as serotonin biosynthesis, indole-pyruvate flux, and DA turnover. These cross-pathway perturbations likely emerge from shared substrate dependencies and competitive enzymatic hierarchies, wherein the depletion of one metabolic sink amplifies pressure on adjacent routes. In particular, diminished serotonin availability can be interpreted as a direct consequence of altered Trp partitioning, whereas DA irregularities appear linked to secondary changes in redox balance and cofactor utilization. Thus, *kat2* deletion should not be conceptualized merely as a KYNA-specific deficit; rather, it reshapes a broader neurochemical network in which serotonergic, dopaminergic, and indole-derived pathways become entrained into a cascade of adaptive yet destabilizing responses.

A key strength of this study lies in the methodological rigor that enabled reliable mapping of subtle, region-specific metabolic shifts. The UHPLC-MS/MS workflow was not only optimized for brain tissue matrices but was applied in a region-resolved fashion, thereby allowing contrasts across CTX, HIPPI, STR, CER, and STEM rather than relying on pooled homogenates [159–161]. Enzyme activities were inferred through calibrated product–substrate ratios, providing functional proxies that extend beyond absolute metabolite abundance [159,161,162]. Importantly, these measurements were embedded in a multi-axis coverage that integrated KYN, serotonergic, indole-derived, and catecholamine pathways. Behavioral assays were performed with a balanced panel that minimizes habituation or training artifacts. Such a design requires a composite technical skill set—ranging from advanced metabolomics and stringent quality control pipelines to expertise in behavioral neuroscience and translational modeling—ensuring robust and reproducible interpretation.

Several limitations should be recognized when interpreting these findings. First, the study design relied on baseline-only testing, which restricts inference on developmental trajectories or dynamic responses to stressors [163–165]. Bulk tissue homogenates were analyzed, inevitably averaging across heterogeneous cell types and masking circuit-specific alterations [163,165,166]. The absence of cell-type resolution is particularly relevant, as astrocytic and neuronal pools may contribute divergently to KYN pathway flux [163,165,166]. Temporal resolution was also limited to a single time point, precluding assessment of circadian phase-dependent or activity-driven fluctuations [163,165,167]. Sex and age effects remain underpowered, raising the possibility that strain differences may emerge in females or in older cohorts [163,165,166]. Likewise, the microbiome context was not systematically profiled, despite its known capacity to shape Trp-indole metabolism. Finally, circadian control was standardized but not manipulated, and phase-dependent metabolic reorganization could shift interpretations [163,165,167].

The neurochemical profile observed in *kat2*^{−/−} mice points toward several therapeutic avenues [67,129,143,168]. Elevated 3-HK alongside reduced XA underscores excessive KMO flux, highlighting selective KMO inhibition as a rational intervention to rebalance the neuroprotective–neurotoxic equilibrium [168,169]. At the same time, the consistent depletion of BH4 and riboflavin-sensitive cofactor pools suggests that restoring the pterin milieu could stabilize DA synthesis and restrain aberrant redox cycling [129,168]. Antioxidant strategies targeting the 3-HK-driven oxidative load may provide additional neuroprotection, particularly in regions where KYNA is diminished [55,129]. Importantly, our data emphasize the need for composite biomarker frameworks that integrate peripheral indices of KYN pathway activity with region-sensitive readouts such as KYNA/QAA ratios, thereby offering translational precision in stratifying patients [55,67,168,170]. These

convergent insights open the path toward mechanism-guided interventions that cut across psychiatric and neurodegenerative spectra [55,67,168].

Moving forward, the central challenge is to shift from descriptive associations toward mechanistic causation [131,171,172]. Stable-isotope-based in vivo flux tracing could clarify how KMO and KAT activities dynamically shape regional metabolite pools under physiological and stress conditions [171,173]. Complementary pharmacologic or genetic rescue experiments, targeting either KMO suppression or KAT reconstitution, will be critical to determine reversibility and compensatory limits of the pathway [128,131,171]. Behavioral paradigms that introduce stress load or learning demands should be layered on top of biochemical profiling to reveal context-dependent vulnerabilities [172–176]. Spatial metabolomics, single-cell resolution analytics, and mesoscale circuit physiology offer the means to connect biochemical imbalances with cellular and network-level adaptations [171,173,176–178]. Finally, microbiome manipulation stands as a tractable lever to probe gut–brain indole inputs [131,171,173,179–181]. Integrating these platforms will close the loop from correlation to causation, refining translational targets across psychiatric and neurodegenerative disease contexts [176,182–184].

KAT II loss establishes a distinctive biochemical landscape marked by a KMO-driven tilt, reduced pterin support, and modulation through indole intermediates. This state disproportionately burdens affective and motor circuits, evident in elevated 3-HK, reduced XA, and region-specific KYNA/QAA shifts, while sparing cognition and sociability under baseline conditions. Such dissociation between neurochemical disequilibria and behavioral resilience highlights the selective vulnerability of motor and emotional nodes. Crucially, these pathway imbalances converge into a coherent, biomarker-ready framework that not only clarifies mechanistic underpinnings of Trp metabolism but also provides a tractable platform for targeted intervention strategies in neuropsychiatric disease contexts.

5. Conclusions

This work integrates region-resolved metabolomics across the STR, CTX, HIP, CER, and STEM with enzyme-ratio proxies, cofactor mapping, and behavioral readouts. A convergent central signature emerges: 3-HK increases and XA decreases pan-regionally, KYNA reduction localizes to CTX and HIP, KMO activity indices rise while KAT III flux falls, and oxidative-stress measures increase broadly, with a hippocampal-specific rise in the excitotoxicity index. Cofactor analyses identify BIO depletion in CER and STEM and BH2 loss in CER. Gut–brain and serotonergic markers also shift, with hippocampal IAA reduction, cortical and cerebellar ICA elevation, and reduced cortical 5-HT turnover, yet cognition, sociability, and coordination remain intact. Consistent with region-specific rewiring rather than a uniform *kat2* effect, we note a paradoxical pattern: striatal KYNA elevation likely reflects compensatory KAT isoforms or astrocyte-mediated KYN shunting; forebrain bias toward AA aligns with oxidative-stress-driven KMO→KYNU flux that diverts 3-HK from KYNA. Taken together, the KAT II/*kat2* loss establishes a reproducible brain–periphery signature (KYNA↓, 3-HK↑, region-tuned KMO/KAT flux) without broad baseline behavioral deficits—best interpreted as evidence of circuit rewiring that buffers output, i.e., whole-axis metabolic disturbance does not immediately translate into behavior. This framing motivates mechanism-targeted interventions (e.g., KMO inhibition, cofactor restoration, antioxidant support) and argues for biomarker-informed stratification. In sum, findings delineate a KMO-tilted, pterin-constrained, indole-modulated milieu that selectively burdens affective and motor subdomains while sparing core cognitive and social functions.

Author Contributions: Conceptualization, Á.S., Z.G., J.T., E.O., L.V., and M.T.; methodology, Á.S., Z.G., E.S., D.M., M.S., A.F-S., Á.F., K.T., K.O., H.I., S.Y., P.M.; software, Á.S., Z.G., E.S., M.S., A.F-S., Á.F., K.O., P.M.; validation, Á.S., Z.G., E.S., M.S., A.F-S., Á.F., K.T., K.O., and P.M.; formal analysis, Á.S., Z.G., E.S., M.S., A.F-S., Á.F., K.O., and P.M.; investigation, Á.S., Z.G., E.S., D.M., M.S., A.F-S., Á.F., K.T., and K.O.; resources, Á.S., Z.G., P.M. and E.O.; data curation, Á.S., Z.G., E.S., M.S., A.F-S., Á.F., K.O.; writing—original draft preparation, Á.S.,

Z.G., and M.T.; writing—review and editing, Á.S., Z.G., E.S., D.M., M.S., J.T., E.O., L.V., and M.T.; visualization, Á.S.; supervision, P.M., J.T., E.O., L.V., and M.T.; project administration, P.M., J.T., E.O., and L.V.; funding acquisition, P.M., J.T., E.O., L.V., and M.T. All authors have read and agreed to the published version of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Funding: Please add: This work was supported by the National Research, Development, and Innovation Office (NKFIH K138125), SZTE SZAOK-KKA (No:2022/5S729), the HUN-REN Hungarian Research Network, the Ministry of Human Capacities, Hungary (grant TKP-2021-EGA-32), the ÚNKP-22-4-SZTE-166 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund, the JSPS Joint Research Projects under Bilateral Programs (Grant Number JPJSBP120203803), and the University of Szeged Open Access Fund, Grant ID: 7571.

Institutional Review Board Statement: The study was conducted in accordance with the Regulations for Animal Experiments of Kyushu University, the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions governed by the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and with the approval of the Institutional Animal Experiment Committees of Kyushu University (A29-338-1 (2018), A19-090-1 (2019)), and approved by the National Food Chain Safety Office (XI./1008/2025, CS/I01/170-4/2022).

Informed Consent Statement: N/A

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Acknowledgments: The figures were created with BioRender (<https://biorender.com> (accessed on 3 September 2025)).

Conflicts of Interest: The authors declare that they have no conflicts of interest and have received no payment for the preparation of their manuscript.

Abbreviations

The following abbreviations are used in this manuscript:

3-HAA	3-hydroxyanthranilic acid
3-HK	3-hydroxykynurenine
3CT	three-chamber test
5-HT	5-hydroxytryptamine (serotonin)
5-HTP	5-hydroxytryptophan
5-HIAA	5-hydroxyindoleacetic acid
AA	anthranilic acid
AADC	aromatic L-amino acid decarboxylase
ALDH	aldehyde dehydrogenase
ARRIVE	animal research: reporting of in vivo experiments
ASD	autism spectrum disorder
BBB	blood-brain barrier
BH2	dihydrobiopterin
BH4	tetrahydrobiopterin
BIO	biopterin
CER	cerebellum
CNS	central nervous system
CTX	cortex
DA	dopamine
DI	discrimination index
DOPAC	3,4-dihydroxyphenylacetic acid
GAD	generalized anxiety disorder
HIPP	hippocampus

IAA	indole-3-acetic acid
ICA	indole-3-carboxylic acid
INS	indoxyl sulfate
KAT	kynurenine aminotransferase
KMO	kynurenine 3-monooxygenase
KYN	kynurenine
KYNA	kynurenic acid
KYNU	kynureninase
L-DOPA	dihydroxyphenylalanine/levodopa
LC-MS	liquid chromatography–tandem mass spectrometry
MAO	monoamine oxidase
MBT	marble burying test
MDD	major depressive disorder
MEL	melatonin
MHPGS	3-methoxy-4-hydroxyphenylglycol sulphate
NMDA	N-methyl-D-aspartate
NORT	novel object recognition test
OBAT	object-based attention test
PI	preference index
QA	quinolinic acid
QAA	quinaldic acid
SCZ	schizophrenia
STEM	brainstem
STR	striatum
Trp	tryptophan
TPH	tryptophan hydroxylase
Tyr	tyrosine
UHPLC-MS	ultra-high-performance liquid chromatography–tandem mass spectrometry
VMA	vanillylmandelic acid
WT	wild-type
XA	xanthurenic acid

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