

Comprehensive behavioral analysis of tryptophan 2,3-dioxygenase (*Tdo2*) knockout mice

Satoko Hattori¹  | Keizo Takao^{2,3}  | Hiroshi Funakoshi⁴ | Tsuyoshi Miyakawa^{1,3} 

¹Division of Systems Medical Science, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi, Japan

²Division of Animal Resources and Development, Life Science Research Center, University of Toyama, Toyama, Japan

³Genetic Engineering and Functional Genomics Group, Frontier Technology Center, Graduate School of Medicine, Kyoto University, Kyoto, Japan

⁴Center for Advanced Research and Education, Asahikawa Medical University, Asahikawa, Japan

Correspondence

Tsuyoshi Miyakawa, Division of Systems Medical Science, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi, Japan. Email: miyakawa@fujita-hu.ac.jp

Funding information

Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan; Grant-in-Aid from Neuroinformatics Japan Center of Japan

Abstract

Aims: Tryptophan 2,3-dioxygenase (TDO2) is an initial rate-limiting enzyme of the kynurenine (Kyn) pathway in tryptophan (Trp) metabolism. The Trp-degrading enzymes, TDO2 and indoleamine 2,3-dioxygenase, are activated by stress and/or inflammation. Dysregulation of Trp metabolism, which causes shifts in the balance between Kyn and serotonin (5-HT) pathways, is associated with psychiatric and neurological disorders. In genetic studies, single-nucleotide polymorphisms in the *TDO2* gene were shown to be involved in psychiatric disorders, such as schizophrenia and depression. It has been reported that targeted deletion of the *Tdo2* gene in mice resulted in reduced anxiety-like behavior, enhanced exploratory activity and cognitive performance, and increased levels of Trp and 5-HT in the hippocampus and midbrain. However, the effect of *Tdo2* gene deletion on behavioral phenotypes has not yet been investigated extensively.

Materials & Methods: We conducted tests to further examine the behavioral effects of knockout (KO) of *Tdo2* in mice.

Results: Deletion of *Tdo2* resulted in seemingly lower anxiety-like behavior, higher locomotor activity, and abnormal gait pattern in mice, though none of them reached study-wide statistical significance. *Tdo2* deficiency had no significant effects on other behaviors, such as prepulse inhibition, and depression-like and social behaviors.

Discussion and Conclusion: The lack of clear phenotypes in *Tdo2* KO mice in this study might be due to the absence of stress and inflammatory conditions, which could induce expression of *Tdo2* mRNA. Further studies are necessary to elucidate the roles of *Tdo2* in behavioral phenotypes related to psychiatric disorders.

KEYWORDS

comprehensive behavioral test battery, knockout mice, kynurenine pathway, tryptophan 2,3-dioxygenase, tryptophan metabolism

1 | INTRODUCTION

Tryptophan 2,3-dioxygenase (TDO2) is a rate-limiting enzyme in tryptophan (Trp) metabolism, which is one of the initial steps in the

kynurenine (Kyn) pathway. Trp is a precursor to the neurotransmitter serotonin (5-HT), and Trp metabolism by the Kyn pathway generates neuroactive metabolites. Fluctuations in Trp metabolism and the subsequent formation of neuroactive metabolites can induce

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2018 The Authors. *Neuropsychopharmacology Reports* published by John Wiley & Sons Australia, Ltd on behalf of The Japanese Society of Neuropsychopharmacology.



physiological changes and potentially induce pathological states, such as psychiatric and neurological disorders.^{1,2} Indeed, human *TDO2* gene polymorphisms are known to be associated with several psychiatric conditions, such as schizophrenia, depression, and attention deficit hyperactivity disorder.^{3,4} Additionally, expression of the Trp-degrading enzymes *Tdo2* and indoleamine 2,3-dioxygenases (*Ido*) mRNA is increased following stimulation of the immune system by lipopolysaccharide (LPS) or polyinosinic-polycytidylic acid (pI:C).⁵ Corticosteroids are known to enhance gene expressions of *Tdo2* and *Ido*.⁶ The Trp-Kyn pathway is widely considered a meeting point of gene and environmental influences in mental disorders.⁷

In previous studies, we identified “immature dentate gyrus (iDG)” phenotype in the adult dentate gyrus (DG). In this phenotype, almost all neurons are arrested in a pseudoimmature state, with regard to molecular and electrophysiological characteristics.^{8–11} The *Tdo2* gene, which has a highly selective expression in the DG, is dramatically reduced in the iDG phenotype. The iDG phenotype has been reported in several strains of mutant mice, including α -CaMKII heterozygous KO mice, Schnurri-2 KO mice, mutant SNAP-25 knock-in mice, and forebrain-specific calcineurin KO mice. These mutant mice exhibited shared behavioral abnormalities including hyperactivity and working memory deficits.^{8–11} Additionally, an iDG-like phenotype was observed postmortem in brains of human patients with schizophrenia and bipolar disorder.¹² This observation suggests the possibility that *TDO2* could be involved in the pathogenesis or pathophysiology of certain psychiatric disorders.

The role of *Tdo2* in the brain has been explored using *Tdo2* knockout mice. Concentrations of Trp and 5-HT in the hippocampi of mutant mice were higher than those in the hippocampi of wild-type mice.¹³ Lower anxiety-like behavior in open-field and elevated plus-maze tests was observed in *Tdo2* KO mice.¹³ Deletion of *Tdo2* in mice has also been associated with increased exploratory activities and cognitive performance.^{14,15} To further investigate the effect of *Tdo2* on mouse behavioral phenotypes, we conducted a comprehensive battery of behavioral tests,^{16,17} evaluating many distinct behavioral domains, ranging from sensorimotor function to cognitive function.

2 | METHODS

2.1 | Animals and experimental design

Generation of *Tdo2* KO mice by a gene-targeting technique using R1 ES cell line was reported previously.¹³ To tightly control genetic background, all mice were backcrossed to the C57BL/6Cr mice for at least 8 generations. All behavioral tests were carried out with at least 19-week-old male mice at the start of testing. Raw data on the behavioral test and the information about each mouse are easily accessible on the public database “Mouse Phenotype Database” (<http://www.mouse-phenotype.org/>). Mice were housed in a room with a 12-hour light/dark cycle (lights on at 7:00 AM) with access to food and water ad libitum. Room temperature was kept at $23 \pm 2^\circ\text{C}$. Behavioral testing was performed between 9:00 AM and

6:00 PM. After each trial, the entire apparatus was cleaned with diluted sodium hypochlorite solution to prevent bias due to olfactory cues. Almost all experiments, except measurement of body weight and body temperature, were performed in a sound proof room. To minimize the effects of previous tests on subsequent tests, we performed the behavioral test battery in a specific order, in which the less stressful tests preceded the more stressful tests. In this study, tests were performed in the following sequence: neurological screens and wire hang (GHNS), light/dark transition test (LD), open-field test (OF), elevated plus-maze test (EP), hot plate test (HP), one-chamber social interaction test (SI), rotarod test (RR), Crawley's sociability and preference for social novelty test (CSI), startle response/prepulse inhibition test (PPI), Porsolt forced swim test (PS), gait analysis (GA), Y-maze test (YM), fear conditioning test (FZ), and tail suspension test (TS). Each behavioral test was separated from the other by at least 1 day (Table S1).

2.2 | Comprehensive behavioral test battery

Most of the behavioral tests were performed as previously described,^{18,19} unless otherwise noted.

2.3 | Open-field test

Locomotor activity was measured using an open-field test. Each mouse was placed in the corner of the open-field apparatus (40 × 40 × 30 cm; AccuScan Instruments, Columbus, OH, USA). The center of the floor was illuminated at 100 lux. Total distance travelled (cm), vertical activity (rearing measured by counting the number of photobeam interruptions), time spent in the center area (20 × 20 cm), and beam-break counts for stereotyped behaviors were recorded. Data were collected for a period of 120 minutes.

2.4 | Light/dark transition test

A light/dark transition test was conducted as previously described.²⁰ The apparatus used for this test comprised a cage (21 × 42 × 25 cm) divided into two sections of equal size by a partition with a door (O'HARA & CO., Tokyo, Japan). One chamber was brightly illuminated (390 lux), whereas the other chamber was dark (2 lux). Mice were placed into the dark side of the cage at the start of the experiment and allowed to move freely between the two chambers with the door open for 10 minutes. The total number of transitions between chambers, latency to first enter the light chamber (s), distance travelled in each chamber (cm), and time spent in each chamber (s) were recorded using ImageLD4 software (see Section 2.12).

2.5 | Elevated plus-maze test

An elevated plus-maze test was conducted as previously described.²¹ The elevated plus-maze test apparatus consisted of two open arms (25 × 5 cm, with 3-mm-high ledges) and two closed arms of the



same size with 15-cm high transparent walls (O'HARA & CO.). The arms and central square were made of white plastic plates and were elevated 55 cm above the floor. Arms of the same type were located opposite to each other. The center of the maze was illuminated at 100 lux. Each mouse was placed in the central square of the maze (5 × 5 cm), facing one of the closed arms. Mouse behavior was recorded during a 10-minute test period. The distance travelled (cm), number of total entries into arms, percentage of entries into open arms, and percentage of time spent in open arms were calculated automatically using ImageEP software (see Section 2.12).

2.6 | Porsolt forced swim test

The Porsolt forced swim test apparatus consisted of four Plexiglas cylinders (20 cm high × 10 cm diameter). A nontransparent panel separated the cylinders to prevent mice from seeing each other (O'HARA & CO.). The cylinders were filled with water (approximately 23°C) up to a height of 7.5 cm. The top of the rack, which holds cages of mice during the experiment, was illuminated at 100 lux. Mice were placed into the cylinders, and immobility and distance travelled were recorded over a 10-minute test period. Images were captured at one frame per second. For each pair of successive frames, the amount of area (pixels) within which the mouse moved was measured. When the area was below a certain threshold, mouse behavior was judged as "immobile," but if it equaled or exceeded the threshold, the mouse was considered as "moving." The optimal threshold for distinguishing between "immobile" and "moving" states was adjusted based on the amount of immobility measured by human observation. Immobility lasting for <2 seconds was not included in the analysis. Retention tests were conducted 24 hours after training. Data acquisition and analysis were performed automatically, using ImageTS software (see Section 2.12).

2.7 | Startle response/prepulse inhibition test

A startle reflex measurement system (O'HARA & CO.) was used to measure startle response to a loud noise and prepulse inhibition of the startle response. The top of the rack, which holds cages of mice during the experiment, was illuminated at 100 lux. A test session began by placing mouse in a plastic cylinder where it was left undisturbed for 10 minutes. White noise (40 ms) was used as the startle stimulus for all trial types. The background noise level was 70 dB. The peak startle amplitude was used as a dependent variable. The intensity of the startle stimulus was either 110 or 120 dB. The prepulse sound was presented 100 ms before the onset of the startle stimulus, and its intensity was 74 or 78 dB (20 ms). Four combinations of prepulse and startle stimuli were used (74-110, 78-110, 74-120, and 78-120 dB), and a test session consisted of six trial types (eg, two types of startle stimulus only trials, and four types of prepulse inhibition trials). Six blocks of the six trial types were presented in a pseudorandom order such that each trial type was presented once within a block. The average intertrial interval was 15 seconds (range: 10-20 seconds).

2.8 | Social interaction test in a novel environment

In the social interaction test, two mice of the same genotype previously housed in different cages, were placed in a box together (40 × 40 × 30 cm; O'HARA & CO.) and allowed to explore freely for 10 minutes. The center of the field was illuminated at 100 lux. Analysis was performed automatically using ImageSI software (see Section 2.12). The total number of contacts, total duration of active contacts (s), total duration of contacts (s), mean duration per contact (s), and total distance travelled (cm) were measured. Active contact was defined as follows: Images were captured at 3 frames per second, and distance travelled between two successive frames was calculated for each mouse. If the two mice contacted each other and the distance travelled by either mouse was longer than 5 cm, the behavior was considered as an active contact.

2.9 | Crawley's sociability and preference for social novelty test

The test for sociability and preference for social novelty is a well-designed method for investigating complex genetics of social behaviors.^{22,23} The apparatus comprised of a rectangular, three-chambered box, and a lid containing an infrared video camera (O'HARA & CO.). Each chamber was 20 × 40 × 47 cm, and the dividing walls were made from clear Plexiglas, with small square openings (5 × 3 cm) allowing access into each chamber. The center of the field was illuminated at 100 lux. We modified the method described by Moy et al.²² In our experiment, a habituation session was performed in the apparatus for 10 minutes the day before the sociability test, and the wire cages in the lateral compartments were located in a corner. In the sociability test, an unfamiliar C57BL/6J male mouse (stranger 1) that had no prior contact with the subject mouse, was placed in one of the side chambers. The location of stranger 1 in the left or right-side chambers was systematically alternated between trials. The stranger mouse was enclosed in a small, circular wire cage, which allowed nose contact between the bars, but prevented fighting. The cage was 11 cm high, with a bottom diameter of 9 cm and bars spaced 0.5 cm apart. The subject mouse was first placed in the middle chamber and allowed to explore the entire social test box for 10 minutes. The amount of time spent within a 5-cm distance of each wire cage and in each chamber was measured with the aid of a camera fitted on top of the box. After the first 10 minutes, each mouse was tested in a second 10-minute session to quantify social preference for a new stranger. A second, unfamiliar mouse was placed in the chamber that had been empty during the first 10-minute session. This second stranger was enclosed in an identical small wire cage. The test mouse had a choice between the first, already investigated unfamiliar mouse (stranger 1), and the novel unfamiliar mouse (stranger 2). The amount of time spent within a 5-cm distance of each wire cage and in each chamber during the second 10-minute session was recorded. The stranger mice used in this experiment were 8- to 12-week-old C57BL/6J male mice, not littermates. Analysis was performed automatically using ImageCSI software (see Section 2.12).

2.10 | Y-maze test

Y-maze test was performed as previously described.²⁴ Exploratory activity was measured using a Y-maze apparatus (arm length: 40 cm, arm bottom width: 3 cm, arm upper width: 10 cm, height of wall: 12 cm). Each subject was placed in the center of the Y-maze field. The number of entries and alterations was recorded using a modified version of the Image EP software. Data were collected for a period of 10 minutes.

2.11 | Contextual and cued fear conditioning test

To assess fear-related learning and memory, each mouse was placed in a test chamber (33 × 25 × 28 cm) with a stainless-steel grid floor (0.2 cm diameter, spaced 0.5 cm apart; O'HARA & CO.) and was allowed to explore freely for 2 minutes.²⁵ Subsequently, a conditioned stimulus (CS; 55 dB white noise) was presented for 30 seconds, followed by a mild foot shock (2 seconds, 0.3 mA), which served as the unconditioned stimulus (US). Two more CS-US pairings were presented with a 2-minute interstimulus interval. Context test was conducted 1 day after conditioning in the same chamber for 300 seconds on each mouse. A cued test with an altered context was then conducted in a triangular chamber (33 × 29 × 32 cm) made of white opaque plastic, which was located in a different room. Tone stimulus for the cued test (55 dB white noise) was applied for 180 seconds. In each test, freezing percentage and distance travelled (cm) were calculated automatically using ImageFZ software (see Section 2.12).

2.12 | Data analysis

As indicated in each experimental method, some behavioral data were obtained automatically by software (ImageLD, EP, SI, CSI, PS, FS, and TS) based on the public domain NIH Image program and Image J program and modified for each test by Tsuyoshi Miyakawa (available through O'HARA & CO.). Statistical analysis was conducted using StatView (SAS Institute, Cary, NC, USA). Data were analyzed using either a paired *t* test, one-way analysis of variance (ANOVA), or two-way repeated measures ANOVA. For the issue of multiple comparisons in the behavioral test battery, we defined study-wide significance as statistical significance after controlling for the false discovery rate (FDR).²⁶ Nominal significance was defined as one that achieved a statistical significance in an index but did not survive FDR correction. The results after statistical analysis are described in Table S2. Graphical numerical values are expressed as mean ± standard error of the mean (SEM).

3 | RESULTS

3.1 | General characterization in *Tdo2* KO mice

Tdo2 KO mice and their wild-type littermates were subjected to a comprehensive battery of behavioral tests. *Tdo2* KO mice showed

no obvious differences in their physical characteristics. There were no significant differences in body weight (Figure S1A), body temperature (Figure S1B), or neuromuscular strength (Figure S1C,D) between the wild-type and *Tdo2* KO mice. Latency to fall off the rotarod (Figure S2A) and sensitivity to a painful stimulus in the hot plate test (Figure S2B) were not significantly different between genotypes. In gait analysis, there are nominally significant differences in stance width of front paws (Figure S3C), percentage of the swing and propulsion phases (Figure S3G) and paw angle (Figure S3L) of hind paws. *Tdo2* deficiency had no significant effects on the other indices in the analysis (Figure S3).

3.2 | Slightly higher locomotor activity and lower anxiety-like behavior in *Tdo2* KO mice

Locomotor activity of *Tdo2* KO mice was evaluated in an open-field test. There were no significant differences between genotypes in the total distance travelled (Figure 1A), vertical activity (Figure 1B), time spent in the center area (Figure 1C), or stereotypic behaviors (Figure 1D). In accordance with the open-field test, there were no significant differences between *Tdo2* KO and wild-type mice in total distance travelled during the light/dark transition test (light box, $F_{1,36} = 0.037$, $P = .8492$; dark box, $F_{1,36} = 1.493$, $P = .2297$), the elevated plus-maze test ($F_{1,36} = 0.361$, $P = .5517$), the social interaction in a novel environment test ($F_{1,17} = 0.495$, $P = .4914$), or Crawley's sociability and social novelty preference tests ($F_{1,36} = 0.256$, $P = .6162$, and $F_{1,36} = 0.002$, $P = .9654$, respectively). By contrast, in mutant mice, the total number of Y-maze entries was larger (Figure 5D), and total distance travelled in the Y-maze was longer (Figure 5G) than wild-type mice.

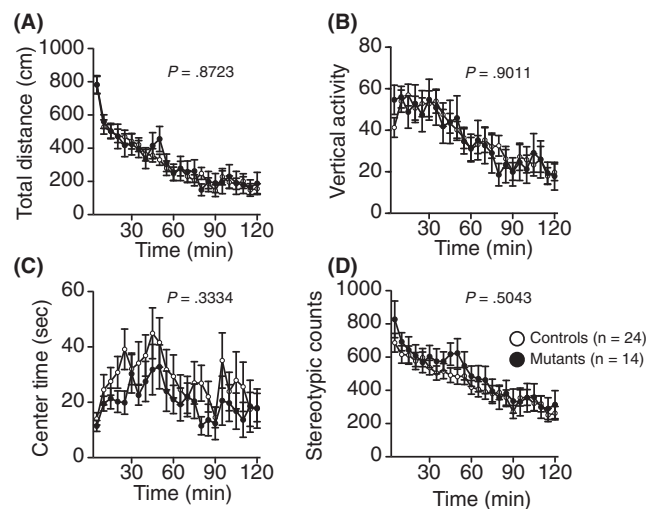


FIGURE 1 Normal locomotor activity of *Tdo2* KO mice in the open-field test. A, Distance travelled in the open-field test was significantly increased in the *Tdo2* KO mice compared with wild-type mice. Counts of vertical activity (B), time spent in the center of the compartment (C), and counts of stereotypic behavior (D) were recorded. Data are presented as means ± SEM for the indicated numbers of animals. The *P* values indicate genotype effect in two-way repeated measures ANOVA

We performed light/dark transition and elevated plus-maze tests in *Tdo2* KO mice. In the light/dark transition test, the total number of transitions, which is a well-validated index of anxiety-like behavior in mice,²⁶ was larger in *Tdo2* KO mice at a nominally significant level (Figure 2A). There were no significant differences between genotypes in the first latency to enter the chamber (Figure 2B) or time spent ($F_{1,36} = 0.51$, $P = .4799$) in the light chamber. On the other hand, there were no significant differences in the time spent in the central area of the open field (Figure 1C) or in any of the parameters examined in the elevated plus-maze test (Figure 2C,D) between *Tdo2* KO and wild-type mice.

3.3 | Normal depression-like behavior and prepulse inhibition in *Tdo2* KO mice

Depression-like behavior of *Tdo2* KO mice was assessed using the Porsolt forced swim and tail suspension tests. There were no significant differences in the percentage of immobility time at Day 1 and Day 2 between the mutant and wild-type mice in the Porsolt forced swim test (Figure 2E). In the tail suspension test, a two-way repeated measures ANOVA showed no significant genotypic effect on immobility (Figure S4).

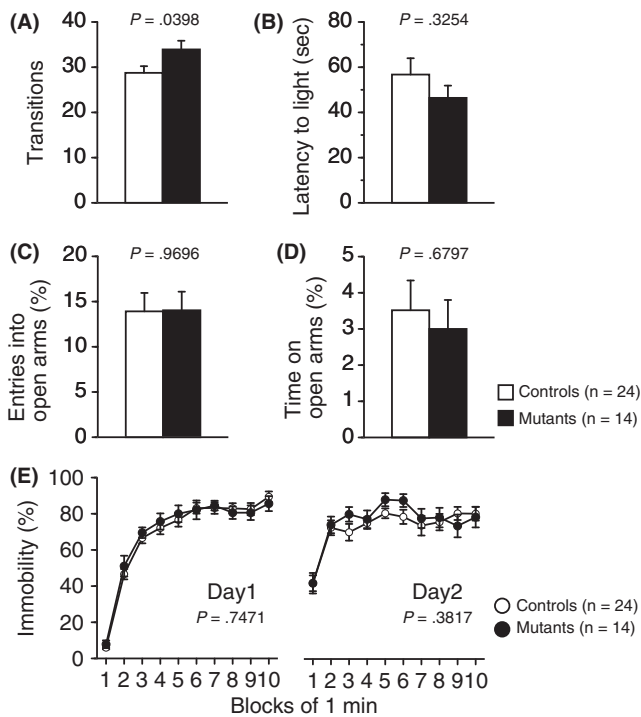


FIGURE 2 Slightly decreased anxiety-like behavior and normal depression-like behavior in *Tdo2* KO mice. A and B, Light/dark transition test: The total number of light/dark transitions (A) and latency to enter the light compartment (B) were recorded. C and D, Elevated plus maze: Percentage of entries into the open arms (C) and percentage of time spent on the open arms (D) were recorded. E, Porsolt forced swim test: The percentage of immobility time for day 1 and day 2 were recorded. Data are presented as means ± SEM for the indicated numbers of animals. The *P*-values indicate the genotype effects in one-way ANOVA (A-D) and two-way repeated measures ANOVA (E)

There was no significant difference in acoustic startle responses between genotypes (Figure 3A). We observed no significant differences in prepulse inhibition between *Tdo2* KO mice and wild-type mice (Figure 3B).

3.4 | Normal social behavior in *Tdo2* KO mice

We subjected *Tdo2* KO mice to two different experiments for social behavior. First, social behavior of *Tdo2* KO mice was evaluated in the test for social interaction in a novel environment. In this assessment, there were no significant differences between genotypes in the total duration of contacts, total number of contacts, or mean duration per contact (Figure 4A-C). We employed Crawley's three-chamber social approach test, which consists of a sociability test and a social novelty preference test in *Tdo2* KO mice. In the sociability test, social behavior can be assessed based on the time spent around a wire cage with an unfamiliar mouse (stranger 1 side) vs the time spent around an empty cage (empty side). Both *Tdo2* KO and wild-type mice spent more time on the stranger 1 side than on the empty side (*Tdo2* KO mice; $t = 3.246$, $df = 13$, $P = .0064$, wild-type mice; $t = 6.513$, $df = 23$, $P < .0001$, respectively), and there was no significant genotypic effect on the time spent around cages in the sociability test (Figure 4D). The preference for a novel mouse (stranger 2 side) over an already-acquainted mouse (stranger 1 side) was then tested in the social novelty preference test. In both genotypes, there were no significant differences in time spent around stranger 1 side vs stranger 2 side (*Tdo2* KO mice; $t = 0.954$, $df = 13$, $P = .3577$, wild-type mice; $t = 1.663$, $df = 23$, $P = .1099$). There was no significant genotypic effect on the time spent around cages in the social novelty preference test (Figure 4E).

3.5 | Normal cognitive function of *Tdo2* KO mice

Cognitive function was evaluated in the fear conditioning and Y-maze tests. In the fear conditioning test, freezing response in mutant mice gradually decreased in the conditioning phase (Figure 5A).

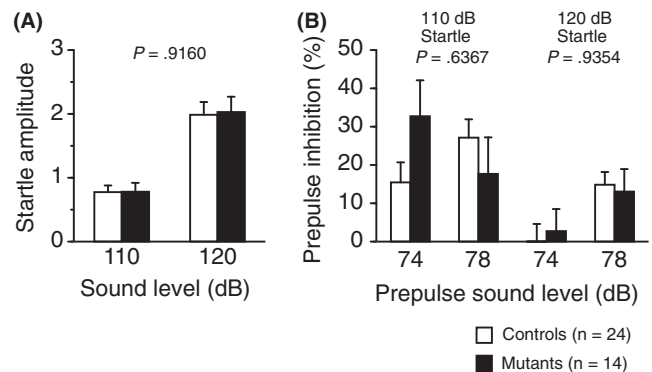
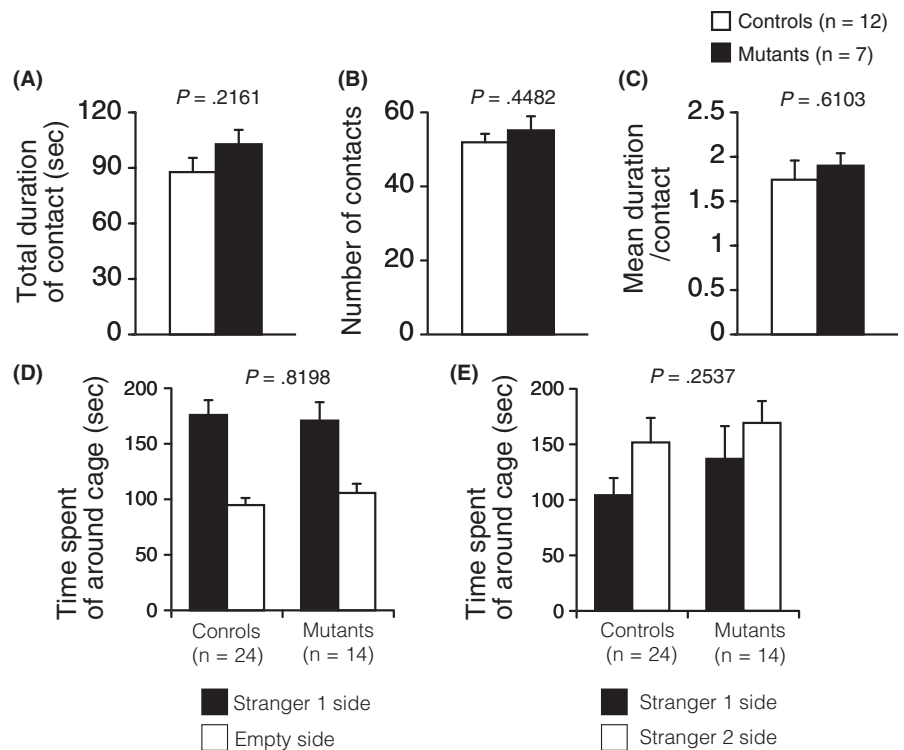


FIGURE 3 Normal prepulse inhibition in *Tdo2* KO mice. Startle amplitude (A) and percent of prepulse inhibition (B) were measured. Data are presented as means ± SEM for the indicated numbers of animals. The *P* values indicate genotype effect in two-way repeated measures ANOVA



FIGURE 4 Normal social behavior in *Tdo2* KO mice. A-C, Social interaction in a novel environment: the total number of contacts (A), total duration of contacts (B), and mean duration of each contact (C) were recorded. D and E, Crawley's three-chamber social approach test: The time spent in the vicinity of the empty cage vs the cage containing a stranger (stranger 1) in the sociability test (D), the time spent in the vicinity of the cage containing a stranger (stranger 2) and the cage containing a familiar (stranger 1) in the preference for social novelty test were recorded. Data are presented as means \pm SEM for the indicated numbers of animals. The *P*-values indicate the genotype effect in one-way ANOVA (A-C) and two-way repeated measures ANOVA (D, E)



There were no significant differences in the distance travelled during and after each foot shock in the conditioning period (Figure S5A). One day after conditioning, the percentage of freezing time in the mutant mice did not significantly differ from that in wild-type mice (Figure 5B). No significant differences in levels of freezing were detected between genotypes before and during the auditory cue in an altered context (Figure 5C). There were no significant differences between genotypes in freezing during contextual testing (Figure S5B) and cued testing, with altered context 2 weeks after the conditioning phase (Figure S5C). To examine short-term spatial working memory, spontaneous alternation behavior in Y-maze was assessed in *Tdo2* KO mice. The percentage of spontaneous alternation in *Tdo2* KO mice did not significantly differ from that in wild-type mice (Figure 5F), although the number of total alternations was significantly larger in the mutant mice (Figure 5E).

4 | DISCUSSION

In the present study, we performed a comprehensive battery of behavioral tests to assess the phenotype of KO mice with a genetic disruption of *Tdo2*. Our results showed lower anxiety-like behavior in *Tdo2* KO mice in the light/dark transition test, but not in elevated plus-maze and open-field tests. The mutant mice also exhibited higher locomotor activity in the Y-maze test and an abnormal gait pattern during gait analysis. These phenotypes achieved only nominal statistical significance. There were no significant effects of *Tdo2* deletion on other behaviors, such as prepulse inhibition, and depression-like, social, and cognitive behaviors.

Tdo2 KO mice exhibited lower anxiety-like behavior, which is consistent with previous study.¹³ As previously reported, mutant mice had increased brain Trp and 5-HT, which was associated with accelerated neurogenesis in the hippocampus.¹³ 5-HT/5-HT_{1A} receptor-mediated neurogenesis has been implicated in reduced anxiety-like behavior,²⁷ suggesting the possibility that the increased serotonergic tone is involved in anxiolytic-like behavior in *Tdo2* KO mice.

In the current study, *Tdo2* KO mice showed lower anxiety-like behavior in the light/dark transition test, but not in the elevated plus-maze or open-field tests. These discrepancies might be due to differences in the number of backcross generations or different experimental conditions. *Tdo2* KO mice were generated using the R1 ES line, which was originally derived from a male blastocyst hybrid of two 129 substrains of mice. In the present study, *Tdo2* mutant and wild-type mice were obtained by intercrossing heterozygotes, after backcrossing with wild-type C57BL/6C mice for 8 generations. The backcross procedure was repeated 5 times in the previous study.¹³ Genetic background may have a profound influence on behavioral phenotypes,²⁸ and flanking genes might be responsible for the different phenotypes observed in mutant mice.²⁹⁻³¹ Additionally, experimental and environmental conditions in the present study, such as age, protocols, and housing conditions, were different from those in previous experiments.¹³ Age is one of the critical factors to be considered when interpreting behavioral phenotypes.¹⁹ As the level of *Tdo2* mRNA gradually increased in an age-dependent manner,³² the influence of *Tdo2* deficiency on behavioral phenotypes might become stronger with age. Indeed, *Tdo2* KO mice showed higher locomotor activity in Y-maze (28-33 weeks old), but not in the open-field test (19-24 weeks old). In addition, open-field and

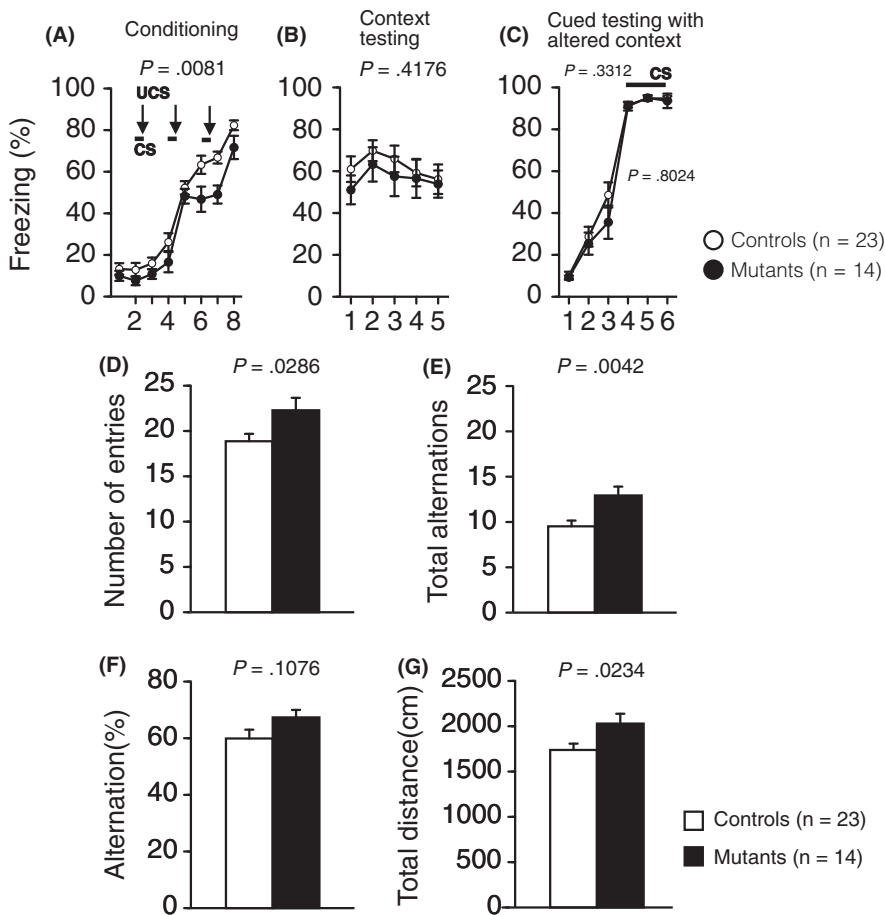


FIGURE 5 Normal cognitive function and increased activity of Y-maze test in *Tdo2* KO mice. A-C, Fear conditioning test: The percent of immobility of conditioning test (A), context test (B), and cued test with altered context (C) were calculated. D-G, Y-maze: The total number of entries (D), the total number of alternation (E), percent of the alternation (F), and total distance travelled (G) were measured. Data are presented as means \pm SEM for the indicated numbers of animals. The *P*-values indicate the genotype effect in two-way repeated measures ANOVA (A-C) and one-way ANOVA (D-G)

elevated plus-maze tests were conducted in 13- to 15-week-old mice in a previous study,¹³ while our behavioral tests were carried out with at least 19 weeks old mice at the start of testing. As *Tdo2* KO mice displayed only slight changes in anxiety-like behavior and locomotor activity, the difference in these conditions might have influenced behavioral phenotypes of the mutant mice.

There were nominally significant differences in stance width of front paws, percentage of the swing and propulsion phases, and paw angle of hind paws in *Tdo2* KO mice. These gait parameters have been implicated in posture stability and walking speed.³³ There is a possibility that *Tdo2* deficiency might lead to changes in motor performance, including locomotor activity. Further studies are needed to confirm the phenotype and to identify their underlying mechanisms.

Tdo2 mRNA has a highly selective expression in the DG of adult normal mice. We previously found that several strains of mice with iDG demonstrated quite a low expression of *Tdo2* mRNA in the adult DG.⁸⁻¹¹ These strains of mice exhibited aberrant behavioral phenotypes comparable to those present in certain human psychiatric disorders, such as hyperactivity, abnormal anxiety-like behavior, and impaired working memory.⁸⁻¹¹ The results of these studies suggest that a low expression of *Tdo2* mRNA in the DG may cause the aberrant behavioral phenotypes. In the present study, however, *Tdo2* KO mice displayed slightly higher locomotor activity, lower anxiety-

like behavior, and normal cognitive function. Our results suggest that *Tdo2* alone is not responsible for the profound behavioral abnormalities observed in mutant mice with iDG, although higher locomotor activity and lower anxiety-like behavior could be partially explained by the lower *Tdo2* mRNA expression level.

It has been reported that inflammation is associated with the induction of the Kyn pathway, which plays an important role in the development of psychiatric disorders.² The expression of *Tdo2* in rodents is increased following treatment with pro-inflammatory mediators, such as LPS or pl:C⁵ and stress hormones.⁶ These inflammatory challenges can also induce depression-like behavior such as helplessness/despair and anhedonia.^{34,35} Additionally, immunoreactivity and mRNA expression of *TDO2* are elevated in the frontal cortex and anterior cingulate gyrus of patients with schizophrenia or bipolar disorder.^{36,37} Collectively, these studies show that a perturbation in Trp-degradation could influence pathological states of neurological and psychiatric disorders. In *Tdo2* KO mice, clear phenotypes may not be evident in this study due to the absence of stress and inflammatory conditions. Indeed, *Tdo2* KO mice exhibited decreased percentage of freezing time immediately after electric foot shocks in fear conditioning test, and increased locomotor activity in Y-maze after a few stressful behavioral tests, including Porsolt forced swim test. It is possible that prior exposure to a stressful condition could

augment behavioral phenotypes in *Tdo2* KO mice. Further studies are necessary to elucidate the behavioral significance of *Tdo2* under such conditions.

ACKNOWLEDGEMENTS

We thank Keiko Toyama of Fujita Health University for their technical support in this study. This work was supported by Grant-in-Aid for Scientific Research on Priority Areas –Integrative Brain Research (Shien)- from the Ministry of Education, Science, Sports and Culture of Japan from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan and a Grant-in-Aid from Neuroinformatics Japan Center of Japan. Behavioral analysis was carried out at Institute for Comprehensive Medical Science, Fujita Health University where behavioral analysis was carried out is accredited as Joint Usage/ Research Center for Genes, Brain and Behavior accredited by MEXT.

CONFLICT OF INTEREST

Dr. Tsuyoshi Miyakawa received research grants from Astellas Pharma Inc. Other authors have no conflict of interests to declare.

DATA REPOSITORY

Raw data on the behavioral test and the information about each mouse are accessible on the public database "Mouse Phenotype Database" (<http://www.mouse-phenotype.org/>).

ANIMAL STUDIES

All behavioral testing procedures were approved by Institutional Animal Care and Use Committee of Graduate School of Medicine of Kyoto University and Fujita Health University.

ORCID

Satoko Hattori  <http://orcid.org/0000-0002-1047-6454>

Keizo Takao  <http://orcid.org/0000-0002-4734-3583>

Tsuyoshi Miyakawa  <http://orcid.org/0000-0003-0137-8200>

REFERENCES

- Szwarcz R, Bruno JP, Muchowski PJ, Wu H-Q. Kynurenines in the mammalian brain: when physiology meets pathology. *Nat Rev Neurosci*. 2012;13:465–77.
- Erhardt S, Schwieler L, Imbeault S, Engberg G. The kynurenine pathway in schizophrenia and bipolar disorder. *Neuropharmacology* 2017;112(Pt B):297–306.
- Comings DE, Gade R, Muhleman D, et al. Exon and intron variants in the human tryptophan 2,3-dioxygenase gene: potential association with Tourette syndrome, substance abuse and other disorders. *Pharmacogenetics*. 1996;6:307–18.
- Miller CL, Murakami P, Ruczinski I, et al. Two complex genotypes relevant to the kynurenine pathway and melanotropin function show association with schizophrenia and bipolar disorder. *Schizophr Res*. 2009;113:259–67.
- Brooks AK, Lawson MA, Rytch JL, et al. Immunomodulatory factors Galectin-9 and interferon-gamma synergize to induce expression of rate-limiting enzymes of the kynurenine pathway in the mouse hippocampus. *Front Immunol*. 2016;7:422.
- Brooks AK, Lawson MA, Smith RA, Janda TM, Kelley KW, McCusker RH. Interactions between inflammatory mediators and corticosteroids regulate transcription of genes within the kynurenine pathway in the mouse hippocampus. *J Neuroinflammation*. 2016;13:98.
- Oxenkrug GF. Tryptophan kynurenine metabolism as a common mediator of genetic and environmental impacts in major depressive disorder: the serotonin hypothesis revisited 40 years later. *Isr J Psychiatry Relat Sci*. 2010;47:56–63.
- Yamasaki N, Maekawa M, Kobayashi K, et al. Alpha-CaMKII deficiency causes immature dentate gyrus, a novel candidate endophenotype of psychiatric disorders. *Mol Brain*. 2008;1:6.
- Hagihara H, Takao K, Walton NM, Matsumoto M, Miyakawa T. Immature dentate gyrus: an endophenotype of neuropsychiatric disorders. *Neural Plast*. 2013;2013:318596.
- Ohira K, Kobayashi K, Toyama K, et al. Synaptosomal-associated protein 25 mutation induces immaturity of the dentate granule cells of adult mice. *Mol Brain*. 2013;6:12.
- Takao K, Kobayashi K, Hagihara H, et al. Deficiency of Schnurri-2, an MHC enhancer binding protein, induces mild chronic inflammation in the brain and confers molecular, neuronal, and behavioral phenotypes related to schizophrenia. *Neuropsychopharmacology*. 2013;38:1409–25.
- Walton NM, Zhou Y, Kogan JH, et al. Detection of an immature dentate gyrus feature in human schizophrenia/bipolar patients. *Transl Psychiatry*. 2012;2:e135.
- Kanai M, Funakoshi H, Takahashi H, et al. Tryptophan 2,3-dioxygenase is a key modulator of physiological neurogenesis and anxiety-related behavior in mice. *Mol Brain*. 2009;2:8.
- Too LK, Li KM, Suarna C, et al. Behavioral and cognitive data in mice with different tryptophan-metabolizing enzymes knocked out. *Data Brief*. 2016;9:275–87.
- Too LK, Li KM, Suarna C, et al. Deletion of TDO2, IDO-1 and IDO-2 differentially affects mouse behavior and cognitive function. *Behav Brain Res*. 2016;312:102–17.
- Takao K, Miyakawa T. Investigating gene-to-behavior pathways in psychiatric disorders: the use of a comprehensive behavioral test battery on genetically engineered mice. *Ann N Y Acad Sci*. 2006;1086:144–59.
- Takao K, Yamasaki N, Miyakawa T. Impact of brain-behavior phenotyping of genetically-engineered mice on research of neuropsychiatric disorders. *Neurosci Res*. 2007;58:124–32.
- Nakao A, Miki T, Shoji H, et al. Comprehensive behavioral analysis of voltage-gated calcium channel beta-anchoring and -regulatory protein knockout mice. *Front Behav Neurosci*. 2015;9:141.
- Shoji H, Takao K, Hattori S, Miyakawa T. Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. *Mol Brain*. 2016;9:11.
- Takao K, Miyakawa T. Light/dark transition test for mice. *J Vis Exp*. 2006:104. <https://doi.org/10.3791/104>.
- Komada M, Takao K, Miyakawa T. Elevated plus maze for mice. *J Vis Exp*. 2008:1088. <https://doi.org/10.3791/1088>.
- Moy SS, Nadler JJ, Perez A, et al. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes Brain Behav*. 2004;3:287–302.
- Crawley JN. Designing mouse behavioral tasks relevant to autistic-like behaviors. *Ment Retard Dev Disabil Res Rev*. 2004;10:248–58.
- Watanabe Y, Tsujimura A, Takao K, et al. Relaxin-3-deficient mice showed slight alteration in anxiety-related behavior. *Front Behav Neurosci*. 2011;5:50.



25. Shoji H, Takao K, Hattori S, Miyakawa T. Contextual and cued fear conditioning test using a video analyzing system in mice. *J Vis Exp*. 2014;85.
26. Crawley JN. Exploratory behavior models of anxiety in mice. *Neurosci Biobehav Rev*. 1985;9:37–44.
27. Santarelli L, Saxe M, Gross C, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science*. 2003;301:805–9.
28. Matsuo N, Takao K, Nakanishi K, Yamasaki N, Tanda K, Miyakawa T. Behavioral profiles of three C57BL/6 substrains. *Front Behav Neurosci*. 2010;4:29.
29. Gerlai R. Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? *Trends Neurosci*. 1996;19:177–81.
30. Silva AJ, Simpson EM, Takahashi JS, et al. Mutant mice and neuroscience: recommendations concerning genetic background. Banbury Conference on genetic background in mice. *Neuron*. 1997;19:755–9.
31. Crusio WE. Flanking gene and genetic background problems in genetically manipulated mice. *Biol Psychiatry*. 2004;56:381–5.
32. Kanai M, Nakamura T, Funakoshi H. Identification and characterization of novel variants of the tryptophan 2,3-dioxygenase gene: differential regulation in the mouse nervous system during development. *Neurosci Res*. 2009;64:111–7.
33. Kale A, Amende I, Meyer GP, Crabbe JC, Hampton TG. Ethanol's effects on gait dynamics in mice investigated by ventral plane videography. *Alcohol Clin Exp Res*. 2004;28:1839–48.
34. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci*. 2008;9:46–56.
35. Dantzer R, O'Connor JC, Lawson MA, Kelley KW. Inflammation-associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology*. 2011;36:426–36.
36. Miller CL, Llenos IC, Dulay JR, Barillo MM, Yolken RH, Weis S. Expression of the kynurenine pathway enzyme tryptophan 2,3-dioxygenase is increased in the frontal cortex of individuals with schizophrenia. *Neurobiol Dis*. 2004;15:618–29.
37. Miller CL, Llenos IC, Dulay JR, Weis S. Upregulation of the initiating step of the kynurenine pathway in postmortem anterior cingulate cortex from individuals with schizophrenia and bipolar disorder. *Brain Res*. 2006;1073–1074:25–37.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Hattori S, Takao K, Funakoshi H, Miyakawa T. Comprehensive behavioral analysis of tryptophan 2,3-dioxygenase (*Tdo2*) knockout mice. *Neuropsychopharmacol Rep*. 2018;38:52–60. <https://doi.org/10.1002/npr2.12006>