Behavioral impairments in a mouse model of Kabuki syndrome associated with dopaminergic and neuroinflammatory modulations

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Running title: Mouse model of Kabuki syndrome, brain, and behavior

This is an Author's Accepted Manuscript for Acta Neuropsychiatrica. This version may be subject to change during the production process.

https://doi.org/10.1017/neu.2025.17 Published online by Cambridge University Press

ABSTRACT

Objective: Kabuki syndrome is a rare multisystem congenital disorder characterized by specific facial malformations and several other symptoms, including motor impairments, increased susceptibility to infections, immune mediators' deficits, anxiety, and stereotyped behaviors. Considering the reports of motor impairments in Kabuki syndrome patients, the first hypothesis of the present study was that this motor dysfunction was a consequence of striatal dopaminergic modulation. The second hypothesis was that the peripheral immune system dysfunctions were a consequence of neuroinflammatory processes. To study these hypotheses the mutant *bapa* mouse was used as it is a validated experimental model of Kabuki syndrome. *Methods:* Exploratory behavior, anxiety-like behavior (light-dark test), repetitive/stereotyped behavior (spontaneous and induced self-grooming), and tyrosine hydroxylase (TH), astrocyte glial fibrillary acidic protein (GFAP), and ionized calcium-binding adaptor molecule 1 (Iba1) striatal expressions were evaluated in female adult *bapa* and control mice. *Results:* Female *bapa* mice did not present anxiety-like behavior, but exploratory hyperactivity and stereotyped behavior both in the spontaneous and induced self-grooming tests. Striatal TH, GFAP, and Iba1 expressions were also increased in *bapa* mice. *Conclusion:* The exploratory hyperactivity and the stereotyped behavior occurred in detriment of the striatal dopaminergic system hyperactivity and a permanent neuroinflammatory process.

Keywords: Mutant Mouse Strain; Grooming; Stereotyped behavior; Dopamine; Astrocytes; Microglia.

SIGNIFICANT OUTCOMES

- *Bapa* mice presented exploratory hyperactivity and stereotyped behavior. Striatal TH, GFAP, and Iba1 expressions were also increased in *bapa* mice.
- The behavioral phenotype was result of the dopaminergic system hyperactivity.
- Dopaminergic system modulation was associated to a neuroinflammatory process.

LIMITATIONS

- Molecular studies about dopamine, TH, cytokine levels, and the neuroinflammatory pathway.

https://doi.org/10.1017/neu.2025.17 Published online by Cambridge University Press

INTRODUCTION

Kabuki syndrome is a rare multisystem congenital disorder characterized by specific facial malformations (peculiar face with long or wide palpebral fissures, lower lateral eyelid eversion, arched eyebrows with the lateral third dispersed, prominent ears, depressed nasal tip, and skeletal and dermatoglyphic abnormalities) that resemble the stage makeup used in Kabuki, a Japanese traditional theatrical form (Boniel et al., 2021). Several other symptoms are documented, including poor physical growth, cardiac, gastrointestinal, and renal anomalies, motor and cognitive impairments, increased susceptibility to infections and immune mediators' deficits (Van Laarhoven et al., 2015, Wang et al., 2019). It is also reported anxiety (Kalinousky et al., 2022) and stereotyped behaviors (Sertcelik et al., 2016, Boniel et al., 2021) in Kabuki patients.

To study Kabuki syndrome and its behavioral, brain, immune, and genetic mechanisms, our group developed a mouse model: *bapa* mice. The *bapa* mouse model is a recessive mutant mouse also known as *bate palmas* (BALB/^{bapa}). It arose from N-ethyl-N-nitrosourea (ENU) mutagenesis (Massironi et al., 2006). Genetic sequencing revealed the missense mutation NM_001033276:c.A3865G:p.T1289A in the lysine (K)-specific methyltransferase 2D (Kmt2d) gene on chromosome 15 (Yamamoto et al., 2019). Indeed, mutations with a loss of function in the KMT2D gene in humans are mainly responsible for Kabuki syndrome (Ratbi et al., 2015, Lu et al., 2016). *Bapa* mice also present hyperactivity, sensory and psychomotor impairments, such as hypotonia, and a slight motor coordination dysfunction (Yamamoto et al., 2019, de Oliveira-Higa et al., 2023). Some of these behavioral impairments, such as hyperactivity, are also found during the prepubertal period (Kirsten et al., 2022). The behavioral findings were associated with dopaminergic system modulation, including increased gene expression of the D1 receptor (de Oliveira-Higa et al., 2023) on adults and increased striatal tyrosine hydroxylase (TH) expression on juveniles (Kirsten et al., 2022).

Besides our mouse model of Kabuki syndrome, there is also a model developed by Bjornsson and colleagues with a heterozygous deletion in the gene encoding the Kmt2d, leading to impairment of methyltransferase function: Kmt2d$^{+/\beta Geo}$ mice (Bjornsson et al., 2014). This model is characterized by reduced neurogenesis, hippocampal memory defects (Bjornsson et al., 2014), shortened long bones and ventral bowing of skulls associated with disrupted endochondral ossification (Fahrner et al., 2019), and DNA methylation aberrations in peripheral blood (Goodman et al., 2023). There are other studies in mice that indirectly are

https://doi.org/10.1017/neu.2025.17 Published online by Cambridge University Press

considered experimental models of the Kabuki syndrome. For example, loss of function of one allele for the KMT2A (Mll1) and Kmt2b (Mll2) genes is also related to cognitive, hippocampal, and neurogenesis defects (Kerimoglu et al., 2013, Shen et al., 2014, Jakovcevski et al., 2015). There is currently no reasonable mechanism pointed out for the motor and immune deficits present in the Kabuki syndrome patients.

Considering that most of the behavioral phenotype found in the Kabuki mouse model is associated with motor activity, the hypothesis of the present study is that behavioral impairments found in Kabuki syndrome are consequences of striatal dopaminergic modulation (Cerovic et al., 2013, Prager and Plotkin, 2019). Moreover, the immune dysfunctions found in Kabuki syndrome may be in detriment of neuroinflammatory processes, since overactivity of astrocytes and microglia and other central nervous system components may affect susceptibility to infections and immune mediators expression (Schwab et al., 2014, Ransohoff et al., 2015, Edison, 2024). To evaluate these hypotheses, the *bapa* mouse model was used. Behavioral aspects of Kabuki syndrome were evaluated, such as anxiety, exploratory behavior, and repetitive/stereotyped behaviors. TH, the rate-limiting enzyme of the dopamine biosynthesis pathway (Baker et al., 2003) was studied for the dopaminergic striatal modulation hypothesis. Astrocytic glial fibrillary acidic protein (GFAP, the major protein constituent of glial intermediate filaments in differentiated astrocytes of the central nervous system) (Sofroniew and Vinters, 2010) and ionized calcium-binding adaptor molecule 1 (Iba1, a macrophage/microglial marker) (Kempuraj et al., 2024) were studied for the neuroinflammatory hypothesis.

MATERIAL AND METHODS

Ethics statement

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

The present study was carried out in strict accordance with the recommendations of the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals (GCULA) of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Paulista University, Brazil (Permit Number:

https://doi.org/10.1017/neu.2025.17 Published online by Cambridge University Press

035/17). All efforts were made to minimize suffering, reduce the number of animals used, and utilize alternatives to *in vivo* techniques when available. The experiments were also performed in accordance with good laboratory practice protocols and quality assurance methods.

Animals, groups, and experimental design

Female adult BALB/c and BALB/c^{bapa} (*bapa*) mice (*Mus musculus*) with approximately 100 days of age on the luteal phase of the estrous cycle were used (n=7/group). The luteal phase was confirmed through microscopic analysis of histological sections from the ovary and uterine horns (Akinjiola et al., 2018). Mice were obtained from the Institute of Biomedical Sciences (University of São Paulo, São Paulo, Brazil) and housed at Paulista University (São Paulo, Brazil) under standard conditions. The mice housing, nutritional conditions, and daily handling and care were standard and previously described by our group (Kirsten et al., 2022).

Mice were first evaluated in the light-dark test and immediately for the spontaneous self-grooming behavior. Twenty-four hours later, they were evaluated in the splash test and immediately their brains were processed for immunohistochemical analysis (TH, GFAP, and Iba1). All the experiments were performed between 9:00 and 11:00 AM to minimize the effects of circadian rhythms. All behavioral assays and the immunohistochemical analyses were performed by investigators who were blinded to the treatment groups.

Light-dark test

The light-dark test was performed to evaluate anxiety-like behavior and exploratory behavior as previously described (Kirsten et al., 2020). This model is based on the inherent conflict between the exploratory drive to a novel place and the avoidance of a lit compartment (Campos et al., 2013). The apparatus consisted of an acrylic box (45 x 27 x 20 cm) containing two compartments (separated by a door with 9 x 7 cm): dark room with black walls and floor (17 cm), and a light room, with white walls and floor (26 cm) and illuminated with a white fluorescent lamp (15W, 4100K). Each mouse was individually placed in the center of the light room, facing the wall opposite to the door. The following parameters were evaluated over a period of 5 min: dark side entry latency (s), total times (s) spent in the dark and in the light sides, and total rearing frequency. The testing room, which was isolated from the experimenter was a small room with dim lighting. A video camera mounted above the arena was used to collect the data. The apparatus was washed with a 5% alcohol/water

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solution before placement of the animals to obviate possible biasing effects from odor cues left by previous mouse.

Spontaneous self-grooming behavior

Spontaneous self-grooming behavior was evaluated immediately after a 30-min habituation period, conducted immediately after the light-dark test. Each mouse was individually placed in the center of a clear observation cage (30 x 16 x 19 cm) and after the habituation period the following parameters were evaluated over a period of 30 min: head washing, body grooming, paw/leg licking, and tail/genital grooming total times (s) (Kirsten and Bernardi, 2017). The testing room and cleaning procedures were the same as those used for the light-dark test. A video camera mounted in front of the arena was used to collect the data.

Splash test

Twenty-four hours after the evaluation of the spontaneous self-grooming behavior, the splash test was conducted in the same clear observation cage, according to a previous study (Reis-Silva et al., 2019). The splash test evaluated the induced self-grooming after spraying a 10% sucrose solution on the dorsal coat of each mouse. The same parameters evaluated for the spontaneous self-grooming behavior were evaluated over a period of 5 min: head washing, body grooming, paw/leg licking, and tail/genital grooming total times (s). The testing room, video recording, and cleaning procedures were the same as those used for the spontaneous self-grooming evaluation.

TH, GFAP, and Iba1 analyzes

Immediately after behavioral tests, mice were euthanized (anesthetic overdose, thiopental, 200 mg/kg, i.p.) and their brains were collected and fixed in 10% buffered formalin for 72h for usual histological procedures. Striatum was studied for the immunohistochemical expression of TH, GFAP, and Iba1 proteins (Guimaraes Marques et al., 2019). It is considered a motor system brain area, involved in the modulation of movements, emotions, and cognition (Groenewegen, 2003), and its relation with neuroinflammation (Abg Abd Wahab et al., 2019, Mancini et al., 2021). Striatum was processed for immunohistochemical analysis as previously described (Kirsten et al., 2022). Briefly, four sections (5 µm thick) per rat were made from each striatum. Immunohistochemistry was performed using the chain polymer-conjugated staining method.

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Monoclonal anti-TH antibody (1:1000, Millipore Cat# IHCR1005-6, Chemicon IHC Select Research, Germany), polyclonal rabbit anti-GFAP immunoglobulin (1:100, Agilent Cat# Z0334, Santa Clara, CA, USA), and polyclonal rabbit anti-Iba1 immunoglobulin (1:100; GeneTex Cat# GTX101495, Irvine, CA, USA) were used as primary antibodies according to protocol from the suppliers, followed by the EnVision+ Kit for detection (Agilent Cat# K4011; HRP, Rabbit, DAB+, Santa Clara, CA, USA). Antigen retrieval was achieved by heating the slides in citrate buffer (pH 6.0) at 95^{o}C for 15 min in a steamer. PBS solution instead of the primary antibody was used as the negative control during immunohistochemical staining. The sections were counterstained with Harris hematoxylin and mounted with DPX (06522, Sigma Aldrich, St. Louis, MO, USA). From each individual immunostained section (for TH, GFAP, and Iba1), five-six photomicrographs were taken (40× objective, Nikon E200 microscope, equipped with a Nikon Coolpix digital camera linked to a liquid crystal display monitor, Kanagawa, Japan). Morphometric analysis was performed using the Image Pro-Plus 6 software (Media Cybernetics, Rockville, MD, USA), calibrated with digital color filters regulating red, green and blue bits, in such a way that only immunostained cells were included and the background staining was excluded from the measurement. For TH, GFAP, and Iba1 immunostaining quantification we used the index per area to represent the extent of the area immunostained for TH in neurons, GFAP in astrocytes or Iba1 in microglial cells compared to the total area of the image (being zero, as the complete absence of staining; and 1, as the total staining of the area). For statistical purposes, means of five-six photomicrograph values from each mouse were used as units.

Statistical analysis

Normality was verified using Shapiro-Wilk (W) or Kolmogorov-Smirnov (KS) tests depending on the sample size (alpha=0.05). When necessary, an outlier identification test was applied (ROUT, Q=5%). The Student's *t*-test (unpaired, two-tailed) was used to compare the parametric data between the groups. The Mann-Whitney *U*-test was used to compare the nonparametric data between the groups. The results are expressed as mean±SEM in box & whiskers (min to max, showing all values) graphs. In all cases, the results were considered significant if $p<0.05$.

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RESULTS

In the light-dark test, both groups passed in the normality tests for all the evaluated parameters (**Supplementary Table 1**). None of the anxiety parameters was altered between groups: dark side entry latency (**Figure 1A**), time spent in the dark side (**Figure 1B**), and time spent in the light side (**Figure 1C**). However, female *bapa* mice presented increased rearing behavior, compared with BALB/c data (**Figure 1D**). In other words, *bapa* mice did not present anxiety-like behavior, but increased exploratory behavior.

In the spontaneous self-grooming behavior evaluation, both groups passed the normality tests for all the evaluated parameters (**Supplementary Table 1**). All the evaluated parameters were altered between groups. Specifically, compared with BALB/c data, female *bapa* mice presented increased time spent for: head washing (**Figure 2A**), body grooming (**Figure 2B**), paw/leg licking (**Figure 2C**), and tail/genital grooming (**Figure 2D**). Therefore, *bapa* mice presented increased spontaneous self-grooming behavior.

In the splash test, one of the four parameters did not pass in the normality tests for both groups: tail/genital grooming time (**Supplementary Table 1**). Compared with BALB/c data, female *bapa* mice presented increased time spent for body grooming (**Figure 2F**) and paw/leg licking (**Figure 2G**), but not for head washing (**Figure 2E**) and tail/genital grooming (**Figure 2H**). Therefore, *bapa* mice presented increased induced self-grooming behavior in the splash test.

For the immunohistochemical analysis of TH expression, both groups passed in the normality tests (**Supplementary Table 1**). Female *bapa* mice presented increased striatal TH expression, compared with BALB/c data (**Figures 3A–C**).

For the immunohistochemical analysis of GFAP expression, both groups passed in the normality tests (**Supplementary Table 1**). Female *bapa* mice presented increased striatal GFAP expression, compared with BALB/c data (**Figures 3D–F**).

For the immunohistochemical analysis of Iba1 expression, both groups passed in the normality tests (**Supplementary Table 1**). Female *bapa* mice presented increased striatal Iba1 expression, compared with BALB/c data (**Figures 3G–I**).

DISCUSSION

Although anxiety is not considered a core symptom of Kabuki syndrome (Van Laarhoven et al., 2015, Wang et al., 2019), there is tangible evidence that it should be considered a neurobehavioral feature: a cohort of 60 individuals with molecularly confirmed Kabuki syndrome presents higher anxiety scores than controls (Kalinousky et al., 2022).

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Moreover, patients with a mosaic KMT2D variant were described with similar systemic features and anxiety manifestations (Boniel et al., 2021). Thus, the present study evaluated anxiety-like behavior in the *bapa* mouse model. The light-dark test was chosen for the evaluation of the anxiety-like behavior since it is considered a reliable and popular test for this purpose on mice (Bourin and Hascoet, 2003, Campos et al., 2013).

Presently, none of the evaluated anxiety parameters were affected, i.e., female adult *bapa* mice did not present anxiety-like behavior. Similarly, prepubertal and pubertal *bapa* mice do not present anxiety-like behavior evaluated on the open-field test (Kirsten et al., 2022). Adult *bapa* mice also do not present anxiety-like behavior evaluated on the elevated plus maze test (Oliveira, 2017). Therefore, although anxiety is a secondary symptom of Kabuki syndrome, the *bapa* mouse model consistently did not result in anxiety-like behavior.

However, female *bapa* mice presented increased exploratory behavior demonstrated by increased rearing frequency in the light-dark test. This pattern was previously demonstrated in the *bapa* mouse model using different variations: adult males and females in the open-field test (increased distance traveled, average speed, and rearing) (Yamamoto et al., 2019); adult males in the open-field test observed for four consecutive days (increased locomotion and rearing and decreased immobility) (de Oliveira-Higa et al., 2023); and prepubertal period in the open-field test (increased rearing frequency) (Kirsten et al., 2022), always comparing to their controls. Thereby, *bapa* mice presented locomotory hyperactivity, a symptom described in patients with Kabuki syndrome (Mervis et al., 2005, Sertcelik et al., 2016).

To understand the neurobiological mechanism responsible for the locomotory hyperactivity, striatal TH expression was evaluated. Female adult *bapa* mice presented increased striatal TH expression, indicating increased dopamine synthesis in the striatal dopaminergic system (Baker et al., 2003). A similar result was found in the prepubertal period (Kirsten et al., 2022). Additionally, adult male *bapa* mice present increased gene expression of the striatal D1 receptor (de Oliveira-Higa et al., 2023). Taken together, the behavioral findings were associated with dopaminergic system modulation, i.e., *bapa* mice presented striatal dopaminergic system hyperactivity, which triggered an increased exploratory activity.

Self-grooming of mice was evaluated because it is a behavioral tool used for studies about stereotyped behavior (Crawley, 2012), which is reported in some patients with Kabuki syndrome (Sertcelik et al., 2016, Boniel et al., 2021). Self-grooming is considered a behavioral pattern very sensitive to genetic manipulations and to study genotype differences

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among selected mouse strains (Kalueff et al., 2007) and human neurological disorders (Kalueff et al., 2016). Therefore, the self-grooming behavioral study was applied for the understanding of the mutant mouse *bapa* and the Kabuki syndrome.

A typical grooming chain in rodents is imbedded into different predictable patterns and microstructures, which include head washing, body grooming, paw/leg licking, and tail/genital grooming (Kalueff et al., 2007). Simply assessing the 'amount' of animal total grooming may be insufficient for correct data interpretation and analysis (Kalueff et al., 2007). Self-grooming can be elicited by various environmental conditions, such as under basal conditions, after an acclimation period in the recording chamber and induced artificially, e.g., following misting rodents with water (using spray) (Kalueff et al., 2007). The present study evaluated these two environmental conditions: basal for the spontaneous grooming and the elicited grooming (using a spray – splash test). Both models revealed increased self-grooming in the female *bapa* mice, compared with the controls. However, the spontaneous self-grooming test resulted in more expressive differences than the splash test. Taken together with its natural conception and a less stressful protocol, the spontaneous self-grooming test was considered a reliable test to study the mutant mouse *bapa* and the Kabuki syndrome.

Self-grooming sequencing, chain initiation, and chain completion in rodents is strongly bidirectionally affected by striatal dopaminergic system modulation, including lesions of the dopamine-containing nigrostriatal tract, administration of various dopaminergic drugs, genetic mutations, and psychological stress (Cromwell and Berridge, 1996, Burguiere et al., 2013, Kalueff et al., 2016). For example, dopamine D1 receptor activation by D1 agonists induces excessive grooming (Berridge and Aldridge, 2000). Similarly, transgenic mice with reduced TH immunoreactivity in different brain areas present impaired grooming behavior (Aloe and Fiore, 1997). Thus, the increased TH expression in the striatum of *bapa* mice was hypothesized as the explanation of the pathway responsible for the increased self-grooming and stereotyped behavior. However, further molecular studies should be conduct to reveal if this is the only pathway involved and to deepen the knowledge.

As previously reported, the Kabuki syndrome patients are commonly diagnosed with immune system deficiencies. It is described as a lack of antibodies, loss of memory cells, IgA deficiency, hyper-IgM syndrome, disturbed differentiation of terminal B-cells (humoral immunodeficiency), and some autoimmune diseases (Van Laarhoven et al., 2015, Wang et al., 2019). The hypothesis of the present study was that this immune dysfunction may be in detriment of neuroinflammation, since overactivity of astrocytes and microglia, as well as

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other central nervous system components may affect susceptibility to infections and immune mediators expression (Schwab et al., 2014, Ransohoff et al., 2015, Edison, 2024). As Kabuki syndrome is characterized by immune dysregulation, up to 17% of its patients present immune thrombocytopenia, often associated to other hematological autoimmune diseases, including autoimmune hemolytic anemia, eventually resulting in Evans disease, with recurrent respiratory diseases and chronic lung inflammation (Leonardi et al., 2023). In fact, *bapa* mice presented increased striatal GFAP and Iba1 expressions. Clearing of dopamine excess may also explain astrocyte hyperactivity since these cells perform metabolic, structural, homeostatic and neuroprotective functions (Sofroniew and Vinters, 2010). It is recognized that astrocytes from the striatum and cortex express D1-like (D1 and D5) and D2-like receptors (D2, D3, and D4) and dopamine signaling impacts on astrocyte morphology and gene expression (Corkrum and Araque, 2021). Inflammation induced by activated microglia can directly damage dopaminergic neurons, inhibiting dopamine synthesis, reuptake, and receptor activity (She et al., 2024). Thereby, the increased expression of striatal GFAP and Iba1 revealed a neuroinflammatory process, which was hypothesized as the responsible for the immune dysfunction found in the Kabuki syndrome.

It is important to mention that the increased striatal GFAP and Iba1 expressions was observed in female adult *bapa* mice not challenged with any immunological agent, which reassembled not an acute, but a permanent (chronic) striatal neuroinflammatory process. This is a novel result, considering that the previous study of our group revealed increased GFAP expression in juveniles after a LPS challenge (Kirsten et al., 2022).

Incidentally, a persistent acute neuroinflammation can turn to a chronic neuroinflammation as it accumulates damage, resulting in neuronal degeneration (Abg Abd Wahab et al., 2019). Neuroinflammation is the response of the central nervous system to disturbed homeostasis and typifies several neurological and neurodegenerative diseases, such as Parkinson's disease, Huntington's disease, multiple sclerosis, narcolepsy, and autism (Ransohoff et al., 2015, Abg Abd Wahab et al., 2019, Mancini et al., 2021). Specifically, astroglial mediated inflammation plays a prominent role in the pathogenesis of neurodegenerative diseases, such as dementia and Alzheimer's disease (Edison, 2024). In fact, intracellular signaling pathways are completely controlled by astrocytes during inflammation. Astrocytes and microglia are involved in cellular and molecular functions for degeneration, vascular signaling, and glialneuronal interactions (Abg Abd Wahab et al., 2019).

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Besides the explanation of the peripheral immunological deficits found in the Kabuki syndrome, striatal neuroinflammation could also explain the striatal dopaminergic system hyperactivity (behavior and TH expression) presently found. There is robust evidence of the cross talk between neurotransmitters and neuroinflammation in the striatum in the mediation of motor behavior (Abg Abd Wahab et al., 2019, Mancini et al., 2021). For example, neuroinflammation is found to be involved in the alterations in dopamine neurotransmission, whereby cytokines ultimately lead to decreased dopamine synthesis, thus decreasing dopamine function, which could lead to neurodegeneration (Abg Abd Wahab et al., 2019). Apropos, dopamine can act as both an inhibitory and excitatory neurotransmitter depending upon its location in the brain and which receptor it binds to (Nakamura et al., 2014). Moreover, the striatum acts as one of the main target regions for dopamine involving the regulation of motor functions (Abg Abd Wahab et al., 2019). Therefore, it is plausible that the striatal dopaminergic system hyperactivity in the *bapa* mice is a consequence of a permanent striatal neuroinflammatory process.

The present study evaluated only female mice based on our previous studies. The motor/exploratory hyperactivity of adult *bapa* mice found in the behavioral assessments (increased distance traveled, average speed, and rearing) presents the same statistical difference, comparing male and female *bapa* mice with its respective control (Yamamoto et al., 2019). The same scenario is revealed in the behavioral and brain analyzes of prepubertal and pubertal mice (Kirsten et al., 2022). Therefore, considering that there are no sex-specific effects in the behavioral and brain tests of *bapa* mice, the effort to minimize the amount of animals used in the experiments, and the tendency in neuroscience studies to avoid sex-discrimination (comparing males and females), only female mice were studied in the present study

In conclusion, *bapa* mice did not present anxiety-like behavior, but exploratory hyperactivity and stereotyped behavior. This phenotype occurred in detriment of the striatal dopaminergic system hyperactivity and a permanent neuroinflammatory process.

AUTHOR CONTRIBUTIONS

Conceptualization: TBK; Methodology: TFB SMGM EFB TBK; Validation: TBK; Formal analysis: TBK EFB; Investigation: TFB EFB TBK; Resources: SMGM EFB TBK; Data curation: TBK; Writing (original draft preparation): TBK; Writing (review and editing): TFB SMGM EFB; Visualization: TFB SMGM EFB TBK; Supervision: TBK; Project administration: TBK; Funding acquisition: TBK EFB.

https://doi.org/10.1017/neu.2025.17 Published online by Cambridge University Press

ACKNOWLEDGEMENTS

The authors are grateful to Wilton P. dos Santos and Paulo A. Vedovato for technical support. This research was supported by the Paulista University (UNIP). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

FINANCIAL SUPPORT

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

All data underlying the findings described in the manuscript are fully available without restriction. Data can be accessed by contacting the corresponding author. All relevant data are within the paper and its Supporting Information files.

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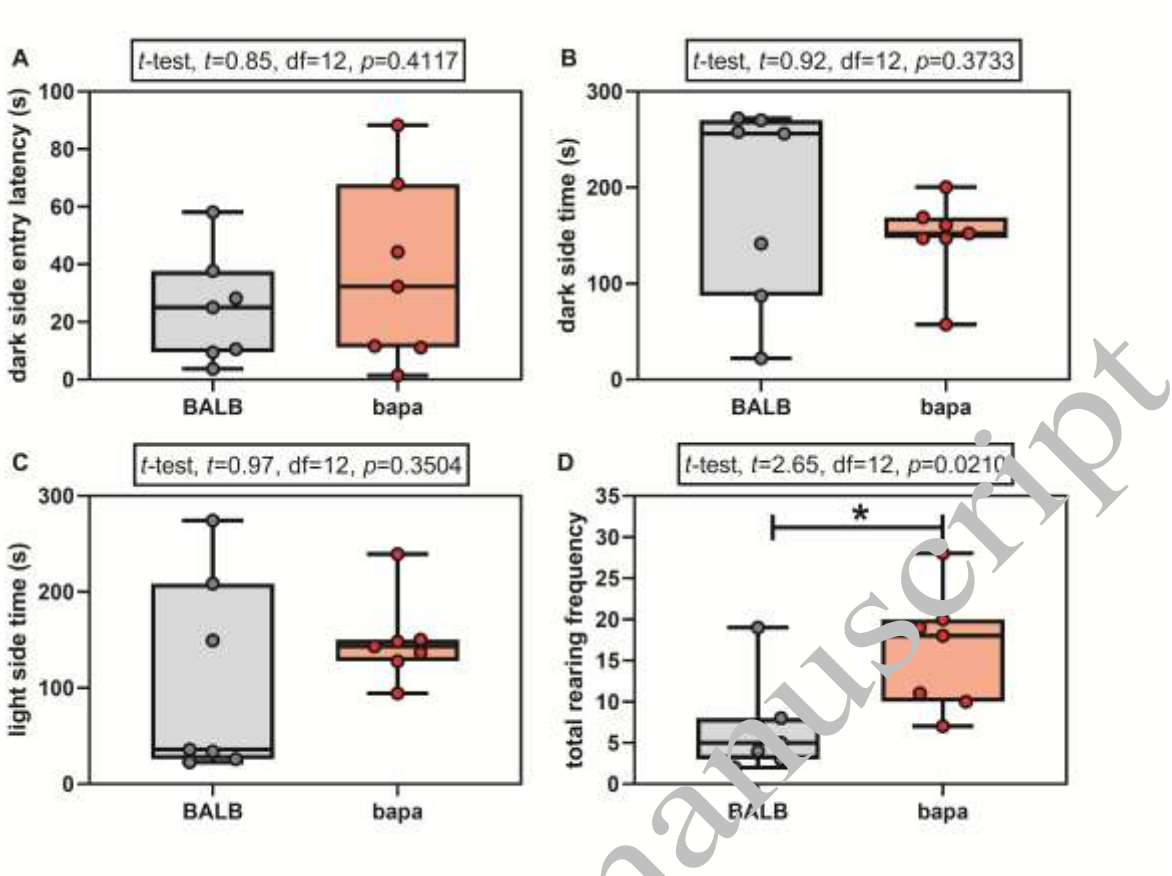

Fig. 1. Light-dark test. Light-dark test of adult BALB/c and BALB/c^{bapa} (*bapa*) mice (n=7 mice per group). *p<0.05 (Student's t-test). Data are expressed as mean ± SEM in box & whiskers (min to max, showing all values) graphs

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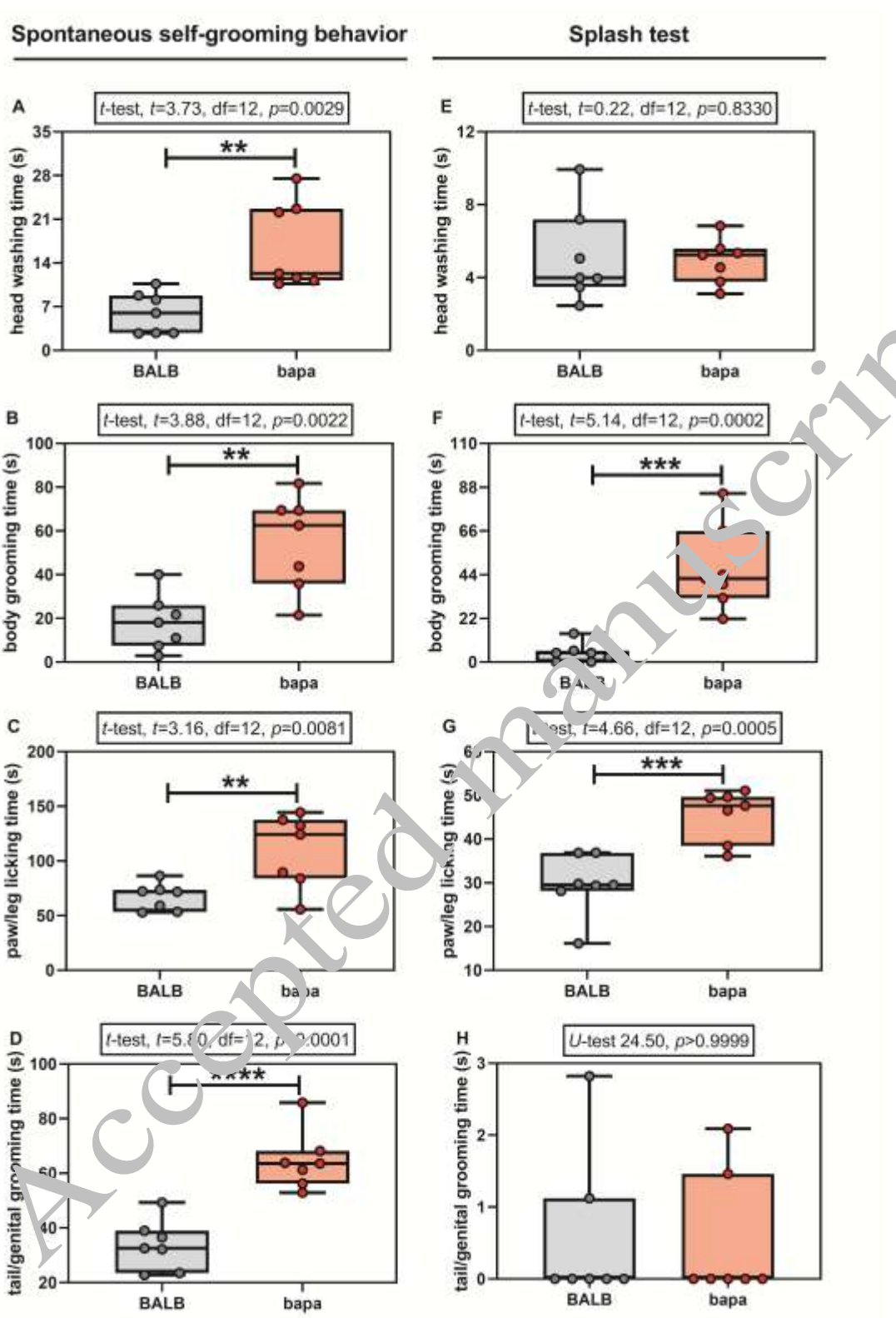

Fig. 2. Self-grooming behavior. Spontaneous and induced (splash test) self-grooming behavior of adult BALB/c and BALB/c^{bapa} (*bapa*) mice (n=7 mice per group). *p<0.05; **p<0.01; ***p<0.001; and ****p<0.0001 (Student's *t*-test and Mann-Whitney *U*-test). Data are expressed as mean ± SEM in box & whiskers (min to max, showing all values) graphs

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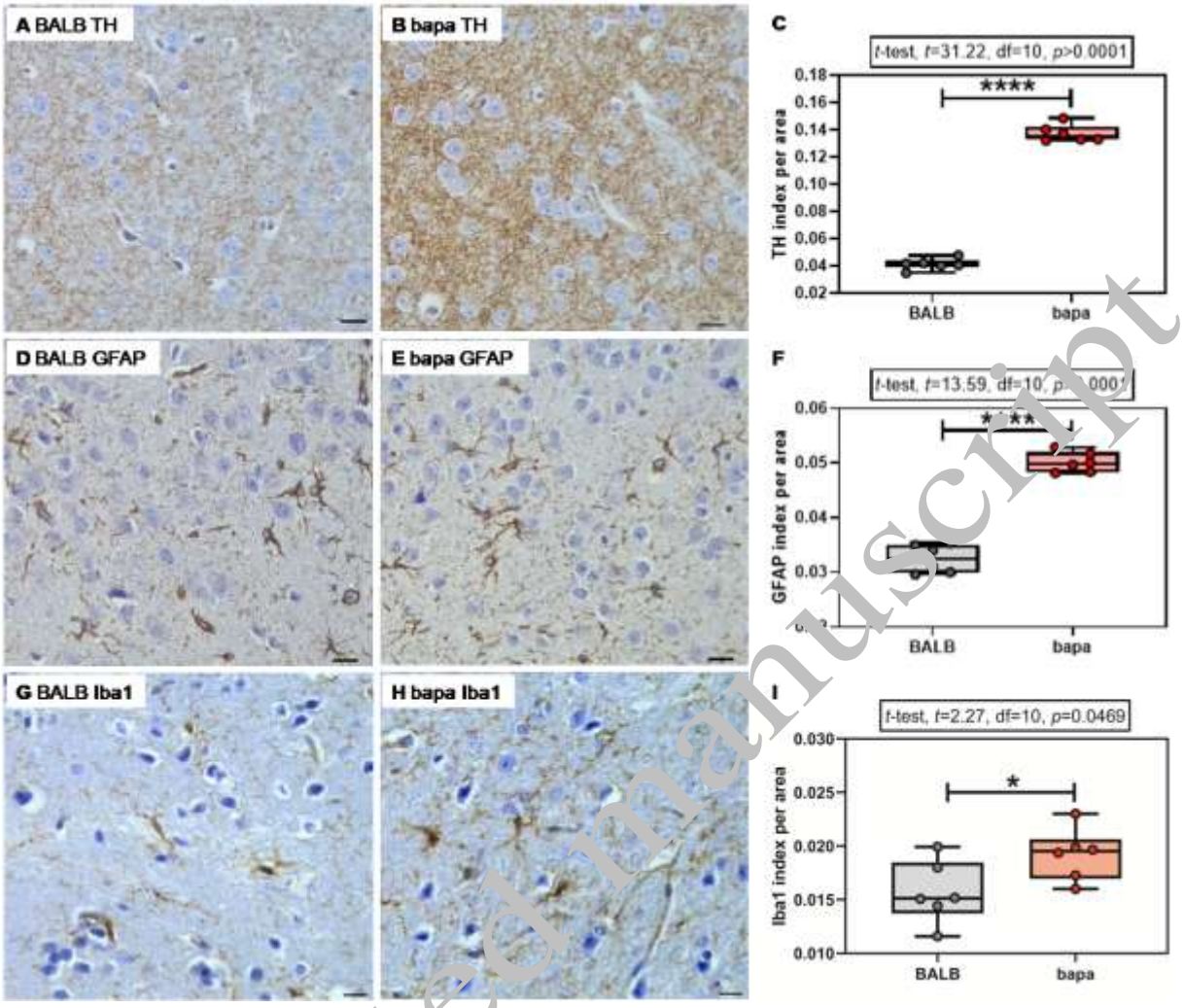

Fig. 3. TH, GFAP, and Iba1. (A–C) Tyrosine hydroxylase (TH), (D–F) astrocyte glial fibrillary acidic protein (GFAP), and (G-I) microglial Iba1 expressions in the striatum of adult BALB/c and BALB/c^{bapa} (*bapa*) mice (n=6 mice per group; 5-6 photomicrographs from each individual brain section). Morphometric analysis from TH, GFAP, and Iba1-immunolabelled sections. Scale bar=50 μm. *p<0.05; **p<0.01; ***p<0.001; and ****p<0.0001 (Student's t-test). Data are expressed as mean ± SEM in box & whiskers (min to max, showing all values) graphs

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