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Research report

Progressive tremor and motor impairment in seizure-prone mutant tremor mice are associated with neurotransmitter dysfunction

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ABSTRACT

Background: The tremor mutant mice present motor impairments comprised of whole-body tremors, ataxia, decreased exploratory behavior, and audiogenic seizures.
Objectives: This study aims to investigate the development of motor dysfunction in this mutant mouse and the relationships with cortical, striatal, and cerebellar levels of GABA, glutamate, glycine, dopamine (DA), serotonin (5-HT), noradrenaline (NOR), and its metabolites. The serum cytokines levels, myelin content, and the astrocytic expression of the glial fibrillary acidic protein (GFAP) investigated the possible influence of inflammation in motor dysfunction.
Results: Relative to wild-type (WT) mice, the tremor mice presented: increased tremors and bradykinesia associated with postural instability, decreased range of motion, and difficulty in initiating voluntary movements directly proportional to age; reduced step length for right and left hindlimbs; reduced cortical GABA, glutamate and, aspartate levels, the DOPAC/DA and ratio and increased the NOR levels; in the striatum, the levels of glycine and aspartate were reduced while the HVA levels, the HVA/DA and 5HIAA/5-HT ratios increased; in the cerebellum the glycine, NOR and 5-HIAA levels increased.
Conclusions: We suggest that the motor disturbances resulted mainly from the activation of the indirect striatal inhibitory pathway to the frontal cortex mediated by GABA, glutamate, and aspartate, reducing the dopaminergic activity at the prefrontal cortex, which was associated with the progressive tremor. The reduced striatal and increased cerebellar glycine levels could be partially responsible for the mutant tremor motor disturbances.

1. Introduction

Tremor is one of the most frequent movement disorders and covers many diseases. Movement disorders include Parkinson's disease, ataxia, dystonia, chorea, Huntington's disease, tics, Tourette syndrome, myoclonus and startle, restless leg syndrome, stiff person syndrome, essential tremor, and gait disorders [1]. In 1998, the International Parkinson and Movement Disorder Society (MDS) changed the concepts of tremor, particularly essential tremor and dystonic tremor [2]. It was discussed that tremor is a common feature in patients with adult-onset focal dystonia and may involve several different body parts and forms of tremor. In addition, recent advances in the field of genetics, suggest that dystonic tremor may even be present without overt dystonia. Monosymptomatic asymmetric rest and postural tremor has been further

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delineated, and apart from tremor-dominant Parkinson's disease, there are several rare conditions including rest and action tremor with normal dopamine transporter imaging without evidence of dopaminergic deficit and essential tremor with tremor at rest. Tremor entities included orthostatic, dystonic, cortical, and thalamic tremors [1,3].

The spontaneous mutation tremor was identified in the Swiss–Webster mouse colony of the laboratory animal facility of the Department of Pathology of the School of Veterinary Medicine and Animal Science at the University of São Paulo, Brazil (FMVZ-USP). These mutant mice displayed normal development but began to present motor impairment comprising whole-body tremors, ataxia, and decreased exploratory behavior after three weeks of age. The severity of the motor impairments seems to be related to aging, suggesting a neurodegenerative profile. Moreover, these mice showed audiogenic generalized clonic seizures. Data demonstrated that audiogenic seizures of tremor mice were also associated with progressive motor impairment and hippocampal alterations of Egr3 and Gabra1 gene expression and amino acid and monoamine content [4]. In addition, alterations of hippocampal Egr3, GABA A receptors, Il-1β, Il6, and Ccl3 expression in audiogenic tremor mice after the seizure was described [5]. Thus, tremor mice were useful for studying neurotransmission pathways as modulators of epilepsy and the pathogenesis of epilepsies with generalized clonic seizures.

The objective of the present study was to assess the behavior profile of tremor mice during infancy, puberty, and adult age compared to wild-type mice (Swiss mice) to characterize the etiology of the mutant motor disturbance. The myelin morphology in several brain areas was investigated because hypomyelination/demyelination could be involved with tremors and spontaneous epilepsy [6]. The astrocytic expression of the glial fibrillary acidic protein (GFAP) was also evaluated because studies showed that astrocytes play an essential role in neuroinflammation in degenerative diseases [7] and epilepsy [8]. Also, peripheral cytokines were measured because, in neurodegenerative disease and epilepsy, they are linked between the peripheral immune system and nervous system dysfunction [9,10]. Lastly, the striatal, cortical, and cerebellar GABA, glycine, aspartate, dopamine (DA), and 5-hydroxytryptamine (5-HT) and their metabolite levels were evaluated in mice because several studies have shown that these neurotransmitters may be involved in tremor [11–14].

2. Material and methods

2.1. Ethics statement

Guide for the care and use of laboratory animals. This study was performed according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. All the procedures were reviewed and approved by the Animal Care Committee of Paulista University (UNIP) (permit no. 035/16), and FMVZ/USP (permit no. 4124150116) conformed to the guidelines of the National Research Council. Committee for updating the guide for the care and use of laboratory animals [15] The experiments followed good laboratory practice protocols and quality assurance methods. All efforts were made to minimize an animal's suffering.

2.2. Animals and experimental groups

A total of 94 male mice – 47 mutant tremor mice and 47 Swiss mice (wild-type mice/WT mice) from the Department of Pathology of the School of Veterinary Medicine, University of São Paulo (Brazil), were used. Control and tremor mice were evaluated at different ages: postnatal days (PND) 15, 45, 60, and 70. All behavior studies were conducted in the laboratory facilities of Paulista University, and the mice were allowed to acclimate to the vivarium for ten days before beginning the experiment.

To obtain the mice of PND15, other adult males and females of both strains were transferred and habituated to Paulista University's facilities and mated. The male offspring of PND15 of different litters were used to perform this experiment. The mice had unrestricted access to filtered and autoclaved water and an irradiated commercial diet (BioBase, Águas Frias, SC, Brazil). Mice were maintained in microisolators (Tecniplast, Buguggiate, VA, Italy) with 4/5 mice/cage under controlled room temperature (22–25 °C) and humidity (55–65 %) with a 12 h/12 h light/dark cycle (lights on at 7:00 A.M.).

2.3. Experimental design

To evaluate the spontaneous behavior and behavior in an inclined platform, seven mutant tremors and seven WT mice of each age were used (Total tremor mice – 21 and total WT mice – 21). The brains and blood for immunohistochemical, morphological, and biochemical studies were made at adult age (PND 60) (08 mice/group). For the motor coordination test, another cohort of mice at PND 70 was used (n = 08 mice per group). For the neurochemical studies, initially ten mice/group were used. All studies were performed between 1 and 5 h p.m. All behavioral procedures were filmed and analyzed blindly by two observers. Data were analyzed by two researchers who were blind to the data. Fig. 1. show the experimental design. Between each mouse observation, all apparatus was thoroughly cleaned using a 5 % alcohol/water solution to eliminate odors from the previous mice.

2.4. Behavioral studies

2.4.1. Spontaneous behavior

Control and tremor mice of different ages were evaluated for spontaneous behavior individually in a glass box (20 × 20 × 9,0 cm) placed in a sound-attenuated room with dim light. For posterior analysis, the behavior was recorded using a video camera (Panasonic HDC-HS20) positioned in front of the apparatus for 3 min. The following parameters were evaluated: 1) time in seconds of movement; 2) time in seconds of restlessness; 3) frequency of rearing with one paw of the mice stood on their hind legs but not in a vertical position in the box; 4) frequency of rearing with two paws of the mice stood on their hind legs but not in a vertical position in the box; 5) full rearing when mice stood on their hind legs with the body in vertical position (90°) in the box; 6) total rearing (sum of all rearing behavior); 7) time in seconds of grooming; and 8) frequency of tail in 45° by hand score.

2.4.2. Behavior in an inclined platform

On the day after observing spontaneous behavior, the same mice of different ages were observed on an inclined platform (45°) with a woven wire floor, and the procedure was recorded for 60 s. The mice were placed at the bottom of the sloped platform, looking up. The latency to reach the top of the platform and the time (sec) for the animal to rotate

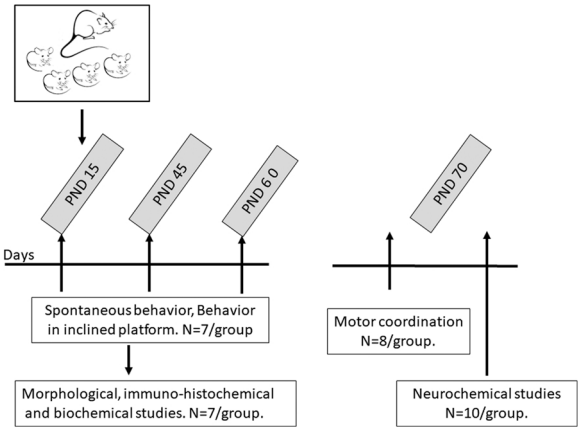

Fig. 1. Experimental design.

the body at 180° were measured for 60 s without any interference.

2.4.3. Motor coordination

The motor coordination (gait analysis) test was performed as described in a previous study [16] at PND70. The gait analysis consisted of a runway apparatus made of acrylic (30 cm long × 5 cm wide × 25 cm high) coated with absorbent filter paper. A dark box was placed at the apparatus's end, allowing the mouse to enter spontaneously and avoid the open area. Mice had their hindlimbs marked with red paint, the right forelimb with black ink, and the left forelimb with blue ink. Furthermore, they were placed to walk on the runway, allowing the marking of the footprints. The test was performed over two days; the first day was used as a training day and habituation to the apparatus, and the second day the performance was evaluated. On both days, only one attempt was allowed. Spatial patterns of the ink prints, including the stride length (the distance between two-foot strikes of the same limb), step length (a limb's forward distance along the direction of travel relative to the contralateral limb), and step width, were measured. Gait symmetry was calculated by dividing the step length by the stride length [17].

2.4.4. Morphological, immunohistochemistry, plasma cytokines, and neurochemical evaluations

Mice were euthanized by decapitation at PND 70 to collect the brain, spinal cord, and blood. Coronal sections were made to reach the frontal cortex, mesencephalon, cerebellum, and spinal cord. These brain regions were chosen due to their involvement in motor control. Brains and spinal cord of the tremor and WT mice were fixed in 10 % buffered formalin for at least 48 h.

2.4.5. Morphological analysis for myelin

Tissue samples were embedded in paraffin for processing for conventional histological procedures. Five-micrometer-thick sections were submitted to luxol fast blue staining to assess the status of the myelin sheaths in the white matter of the mesencephalon, cerebellum, and spinal cord.

2.4.6. Analysis of GFAP in astrocytes by immunohistochemistry

Astrocytic GFAP immunohistochemical procedures using the avidin-biotin-peroxidase complex (ABC) method were performed in the frontal cortex and mesencephalon. Polyclonal rabbit anti-GFAP immunoglobulin (1:1000; Z0334, Dako, Glostrup, Danmark) as the primary antibody and biotinylated secondary antibody (K0690, Dako Universal LSAB 2 System, HRP, Glostrup, Danmark) were used. Five photomicrographs from each frontal cortex and mesencephalon section were made using a 40x objective. The area of astrocytes and their marked brown processes was automatically calculated, in pixels, using Metamorph software (Molecular Devices, Sunnyvale, CA, USA) calibrated with digital color filters that regulated red, green, and blue bits such that only positive cells were included. Background staining was excluded from the measurement.

2.4.7. Plasma cytokines

Blood was centrifuged, and the plasma was processed for cytometry using the BD™ Cytometric Bead Array (CBA) system (Mouse inflammation kit). IL-6, IL-10, MCP-1, IFN- γ, TNF, IL12p70 were measured.

2.4.8. Neurotransmitter evaluations

Tremor and WT male mice at PND 70 were euthanized by decapitation, and the frontal cortex, striatum, and cerebellum were dissected, weighed, and placed on an ice-cold plate. Once assayed, each sample was quickly frozen and stored in an ultra-freezer at − 80 °C. The levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxy indole acetic acid (5-HIAA), noradrenaline (NA) and homovanillic acid (VMA) were determined using high-performance liquid chromatography (HPLC) with a C-18 column and an electrochemical detector (Shimadzu, model 20 A, Kyoto, Japan). DA, 5-HT, and. NA turnover (metabolite/neurotransmitter ratio, i.e., 5HIAA/5-HT, DOPAC/DA, HVA/DA, VMA/NA) was calculated. The detection limit for monoamines was two 0.2 pg, the coefficient of variation was less than 15 %, and the curve linearity was greater than 0.9. The levels of the amino acids GABA, glutamate, glycine, and aspartate were measured by HPLC (HP, model 1100) with a Beckman Ultrasphere ODS-PTH column and sample injector (valve for 1.0 ml), and the limit of detection was 20 pg [4].

2.5. Statistical analysis

Homoscedasticity was verified using the F-test or Bartlett's test, and normality by the Kolmogorov-Smirnov test. An unpaired t-test was used to analyze the spatial parameters in the gait test and the neurotransmitters and their metabolites in the frontal cortex, cerebellum, and striatum. Spontaneous behavior, behavior in an inclined platform, and cytokine levels were analyzed by two-way ANOVA followed by Sidak's multiple comparisons tests. The data are expressed as mean ± SEM, and the results were considered significant at $\alpha < 0.05$. The statistical analyses were performed using GraphPad Prism® software.

3. Results

3.1. Spontaneous behavior

Direct observation of the tremor mice on PND 15, 45, and 60 showed a progressive increase in tremors until adulthood. The tremor mice remained with the body near the ground, and the hindquarters fell slightly.

The time dispended in the movement (Fig. 2a), the rearing with one paw (Fig2c), the partial rearing (Fig. 2d), the full rearing (Fig. 2e), and total rearing (Fig. 2f) were reduced in the tremor mice relative to WT in all ages. Otherwise, the resting time was increased in mutant mice relative to WT in all ages (Fig. 2b). A decreased grooming time (Fig. 2g) and increased tail slope (Fig. 2h) was observed in the tremor mice relative to WT mice at all ages. The two-way ANOVA statistical data are presented in Supplementary Table 1.

3.2. Behavior in the inclined platform

The time of ramp-up (Fig. 3a) and the rotation time (Fig. 3b) increased in the tremor mice relative to the WT mice of all ages. The two-way ANOVA statistical data are presented in Supplementary Table 1.

3.3. Motor coordination

In the motor coordination (gait analysis) test, the tremor mice showed shorter stride lengths for their right and left hindlimbs than WT mice (Figs. 4a and 4d). Additionally, mutants presented shorter step lengths for the right and left hindlimbs (Fig. 4b and e). In contrast, the step width was wider in tremor mice than in WT mice (Fig. 4g). Gait symmetry did not show statistical differences between WT and tremor mice (Fig. 4c and f).

3.4. Myelin and GFAP analysis

No differences in the GFAP-immunolabeled area were observed between the WT and tremor mice in either the frontal cortex or mesencephalon areas (Supplementary Fig. 1). In addition, no apparent differences were noted between the WT and tremor mice regarding the myelin sheaths in the white matter of the mesencephalon, cerebellum, and spinal cord (Supplementary Fig. 2).

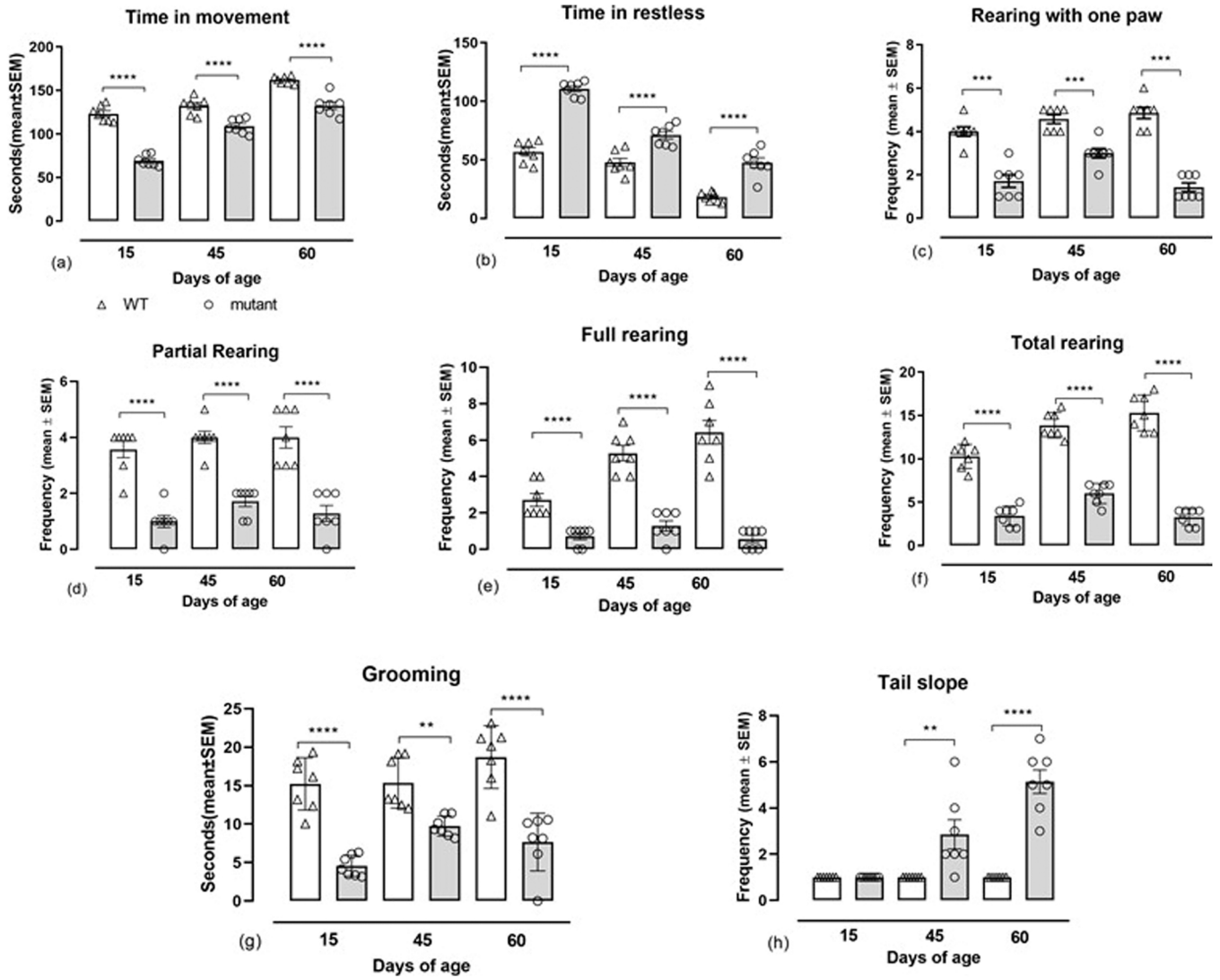

Fig. 2. Spontaneous behavior of mutant and WT mice. a) time of movements in sec.; b) time of restlessness in sec.; c) frequency of rearing with one paw; d) frequency of partial rearing; e) total rearing; f) full rearing; g) total rearing; f) time of grooming, sec; h) frequency of tail slope. Data are presented as means ± SEM of 7 mice/group. Two-way ANOVA followed by Sidak's multiple comparisons tests. ** $p < 0.01$; ***$p < 0.001$ relatives to the WT group.

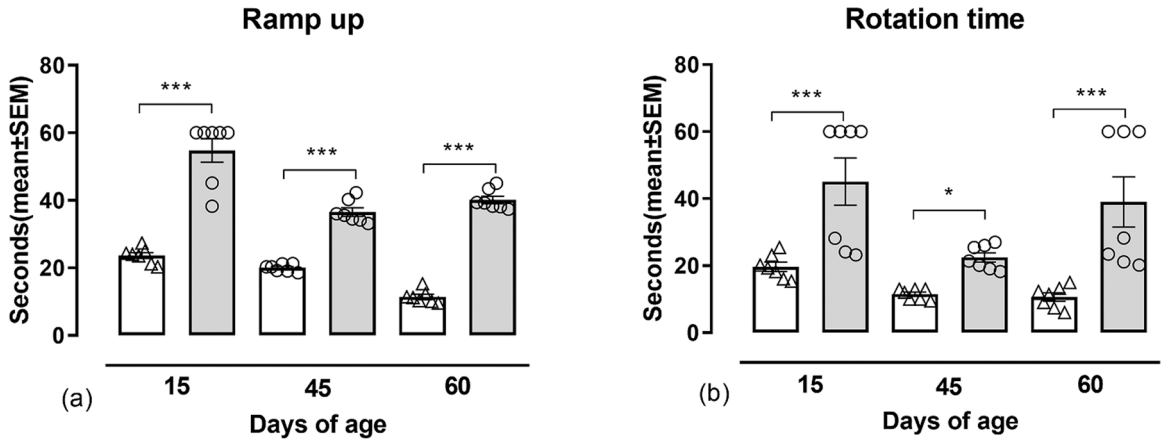

Fig. 3. Time in seconds to ramp up (a) and negative geotaxis (b) of WT and mutant mice at 15, 45, and 60 days of age. Data are presented as the means ± SEM of 7 mice/group. Two-way ANOVA followed by Sidak's multiple comparisons tests. ** $p < 0.01$, *** $p < 0.0001$ relative to the WT group.

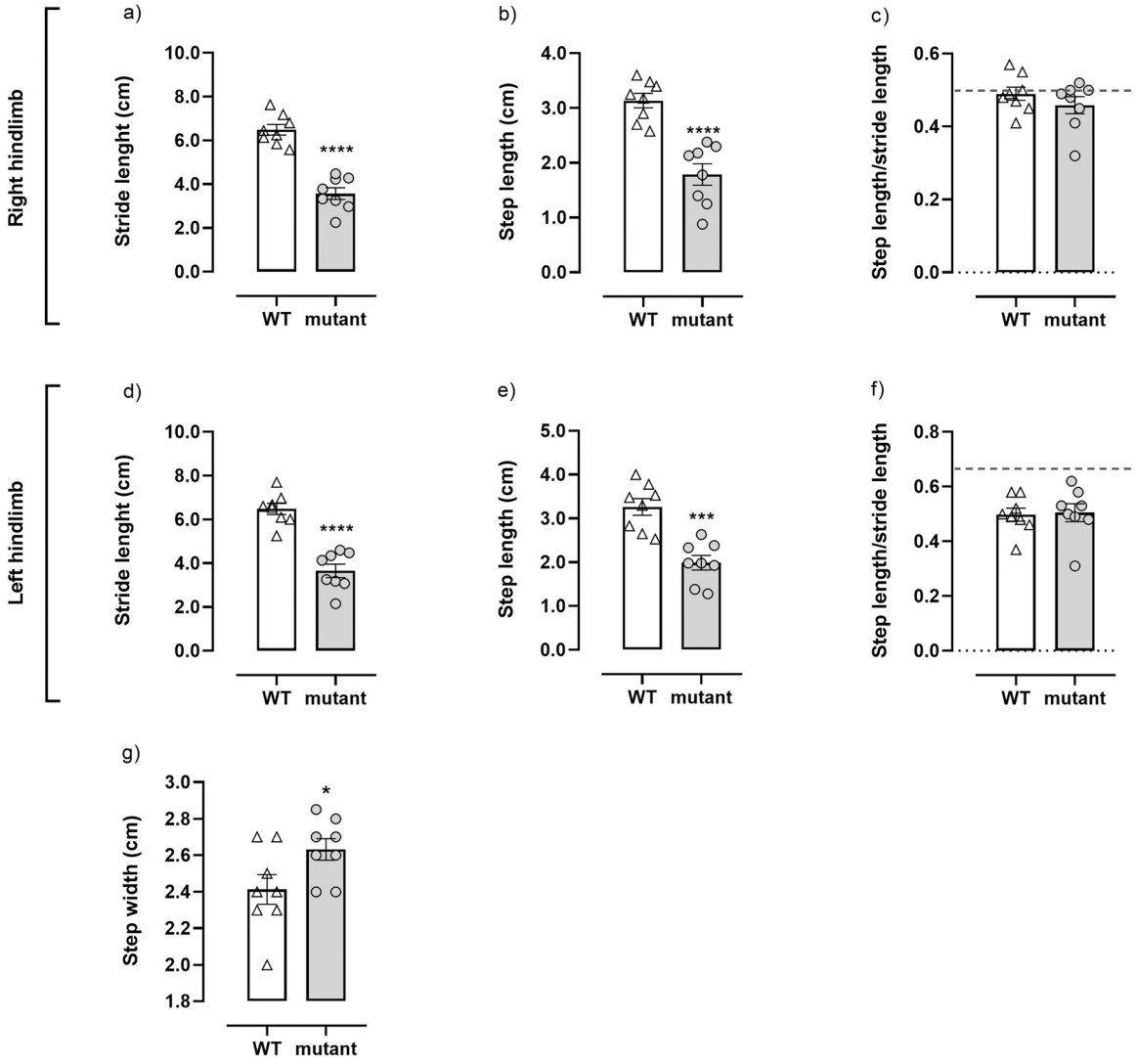

Fig. 4. Spatial parameters in the gait analysis test: right and left hindlimb stride length (a and d); right and left hindlimb step length (b and e); right and left hindlimb gait symmetry (c and f), and step width (g) of WT and mutant mice (n = 8/group). The dotted line indicates a spatial symmetry value of 0.5. Data are presented as the means ± SEM. An unpaired t-test was employed to evaluate strain differences. N = 8/group *p < 0.05, *** p < 0.001,****p < 0.0001.

3.5. Plasma cytokines

The plasma cytokine levels of WT and tremor mice evaluated at different ages were not different. Supplementary Table 2 shows these data.

3.6. Brain neurotransmitter and evaluations

The cortical concentrations of GABA, glutamate, and aspartate, as well as striatal glycine and aspartate levels, were decreased in tremor mice. In the cerebellum, the glycine levels increased (Fig. 5).

When we evaluated the serotoninergic and noradrenergic systems, we observed an increase in the striatal levels of the 5HIAA and of the 5HIAA/5HT ratio in the mutant mice; in the cerebellum, the 5-HIAA levels increased. Also, the tremor mice showed increased levels of NOR in the cerebellum and decreased VMA/NOR ratio in the frontal cortex relative to WT mice. The VMA levels and VMA/NOR were undetectable in the striatum (Fig. 6). Concerning the DA system, the HVA striatal levels of the tremor mice increased, whereas the DOPAC/DA ratio decreased in the frontal cortex; no changes were observed when we analyzed DA, DOPAC, the HVA/DA, and HVA + DOPA/DA ratios in all areas (Fig. 7). All statistical data are shown in Supplementary Table 3.

4. Discussion

Mutant mice with tremor phenotypes are frequently associated with bradykinesia, rigidity, postural instability, and reduced range of motion [18,19]. Others present only tremors [20] or are associated with clonic seizures [21] and severe ataxia [22,23] or whole-body tremors with ataxia [23]. Garcia-Gomes et al. [24] characterized that the novel spontaneous mutation tremor mice are associated with progressive motor impairment and hippocampal alterations of Egr3 and Gabra1 gene expression and amino acid and monoamine content.

In the present study, the decreased spontaneous movements in tremor mice were observed according to age concomitant with increasing resting movements and impaired rearing and grooming behaviors. It is known that mice have a shorter and accelerated early life compared to humans. The infantile age in this experiment corresponds to ~2 years of humans. The PND 45 corresponds to puberty and PND 60 and 70 to adult age [25]. Although the tremor was not quantified, it was present at all ages, increasing from 15 to 60 days.

The grooming behavior requires that the mouse keep the body partially erect with coordinated movements to exhibit the behavior [26]. Thus, the rearing impairment with increased resting could explain the grooming behavior impairment. Moreover, the increased tail at 45°

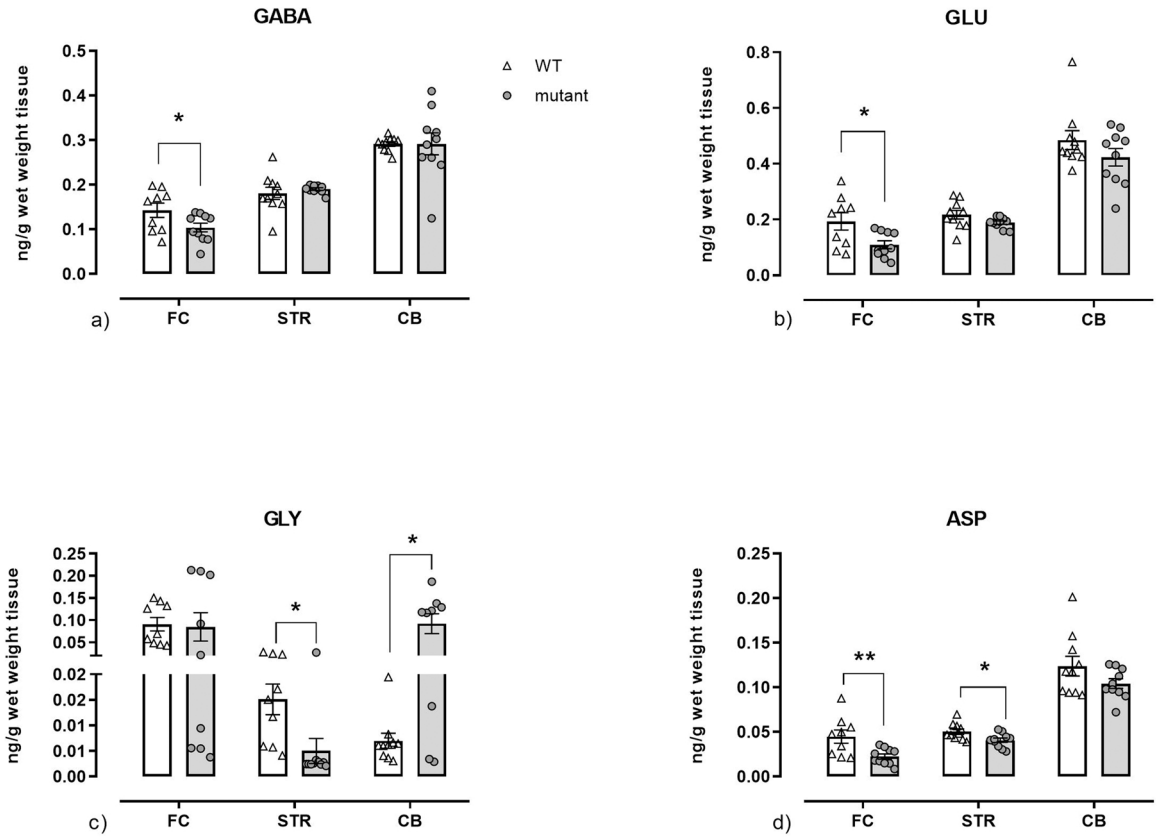

Fig. 5. Neurotransmitter amino acid tissue contents (ng/g wet weight tissue) in the frontal cortex (FC), striatum (STR), and cerebellum (CB) of WT and mutant mice. a) GABA (gamma-aminobutyric acid); b) GLU (glutamate); c) GLY (glycine); and d) ASP (aspartate). Data are presented as the means ± SEM. An unpaired t-test was employed to evaluate strain differences. N = 8–10/group. * $p < 0.05$; ** $p < 0.01$.

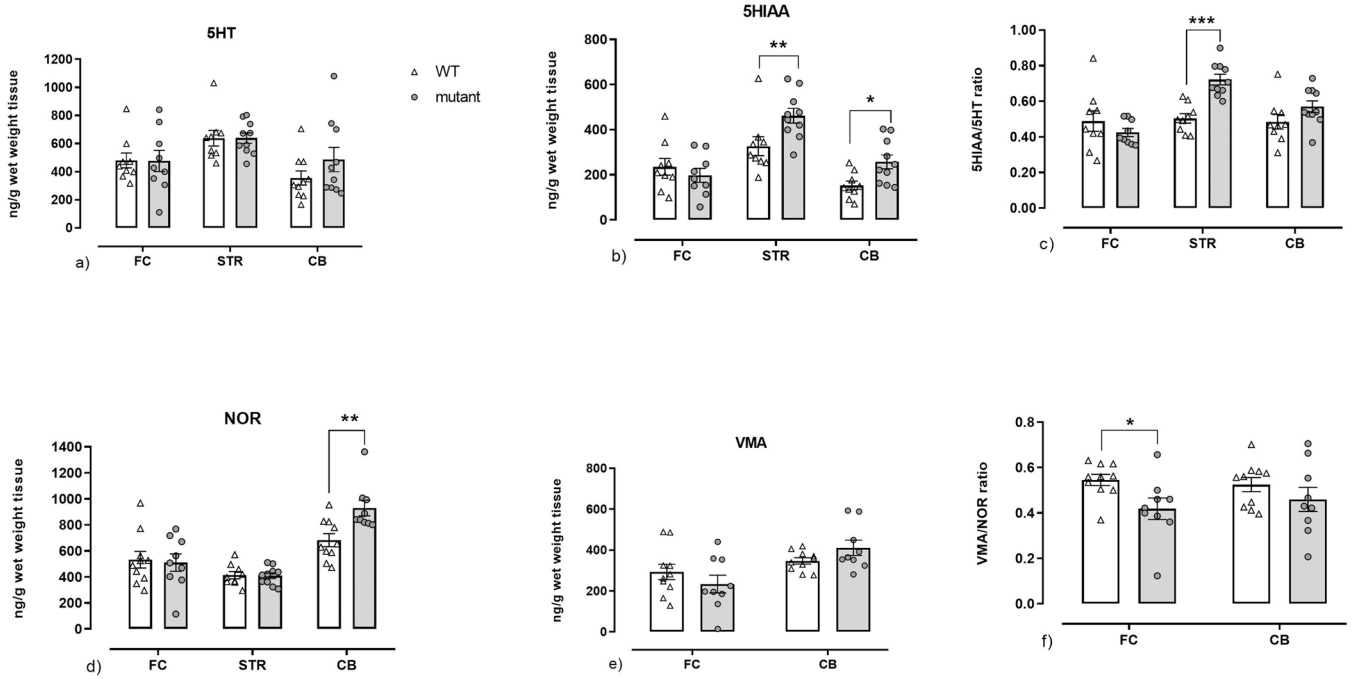

Fig. 6. Serotonin and noradrenaline tissue contents (ng/g wet weight tissue) and turnover rates in the frontal cortex (FC), striatum (STR), and cerebellum (CE) of WT and mutant mice. a) 5HT (5-hydroxytryptamine), b) 5HIAA (5-hydroxyindole acetic acid); c) 5HIAA/5HT ratio; d) NOR (noradrenaline), e) VMA (vanillylmandelic acid), and f) VMA/NOR ratio. Data are presented as the means ± SEM. An unpaired t-test was employed to evaluate strain differences. N = 8–10/group. * $p < 0.05$; **$P < 0.01$; *** $p < 0.001$, **** $p < 0.0001$.

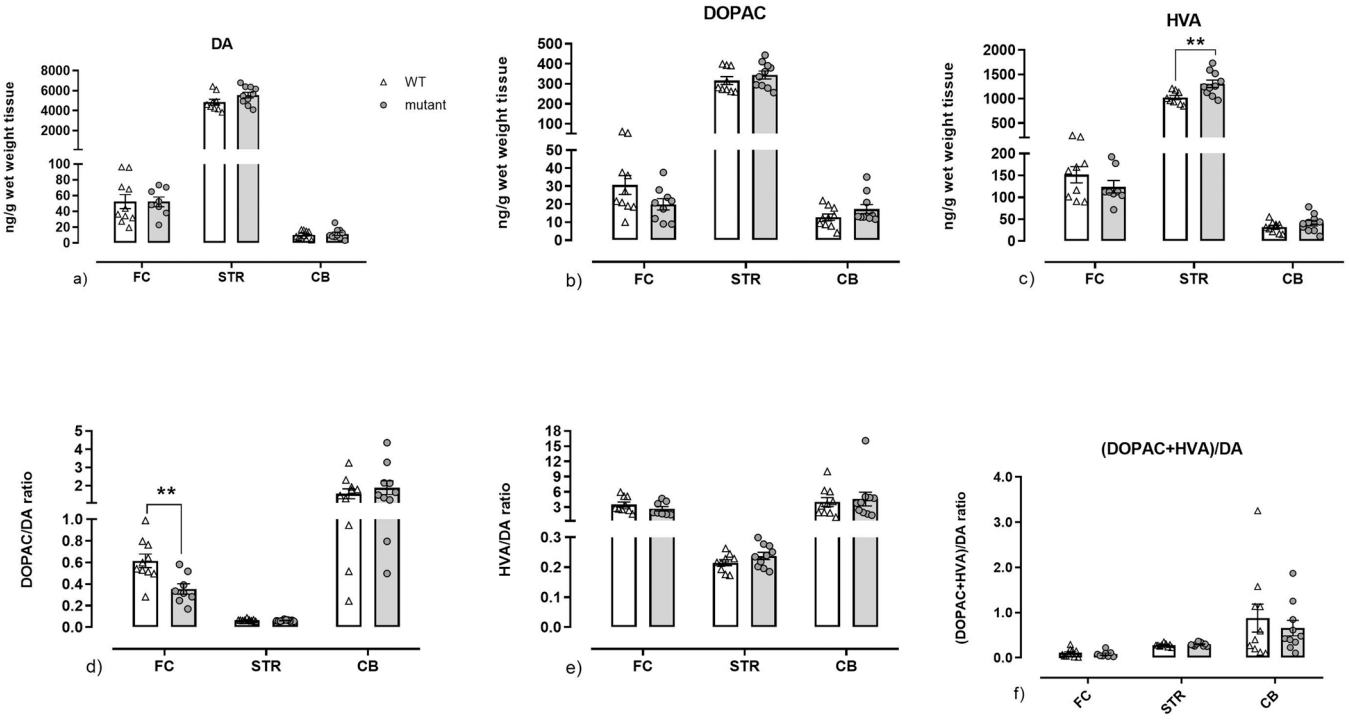

Fig. 7. Neurotransmitter monoamine tissue contents (ng/g wet weight tissue) and turnover rates in the frontal cortex (FC), striatum (STR), and cerebellum (CE) of WT and mutant mice. a) DA (dopamine); b) DOPAC (3,4-dihydroxyphenylacetic acid); c) HVA (homovanillic acid), d) DA/DOPAC ratio; e) HVA/DA ratio, and f) DOPAC + HVA/DA ratio. Data are presented as the means ± SEM. An unpaired t-test was employed to evaluate strain differences. N = 8–10/group. * $p < 0.05$; **$p < 0.01$; *** $p < 0.001$, **** $P < 0.0001$.

of the mutant mice relative to WT mice suggests an attempt to maintain the posture. This scenario strongly suggests that the mutant mice had bradykinesia associated with postural instability and a reduced range of motion.

In the present study, a delay in the behavior in an inclined platform agrees that tremor mice present bradykinesia and a reduced range motion because they showed a progressive increase in the time to reach the top of the platform. Additionally, the increased time to rotation is consistent with motor impairments in this mutant mouse.

The rearing behavior and rotation time associated with the increase in the tail elevation strongly suggest an impairment of the development of dynamic postural adjustments and imply the integrity of muscular and motor function [22,27]. Additionally, the longer time to attain the top of the ramp could reflect the incapacity of the mutant mice to initiate a voluntary movement, which could constitute a possible sign of akinesia [28]. These motor impairments were observed until adult age, suggesting a neurodegenerative profile.

Genetic manipulations, brain injury, neurodegenerative diseases, and pharmacological treatments can alter motor skills in mice. To confirm the motor impairment of the mutant mice in adult age, we analyzed its motor coordination in the beam walking assay. The beam walking assay can assess fine motor coordination and balance [29]. Locomotion in normal mice comprises an organized sequence of coordinated movements in the four limbs and posture and balance in synchronized order. This behavioral model is beneficial for detecting subtle deficits in motor skills and balance that other motor tests may not detect.

Hind limb stride length positively correlates with animal velocity and femur size. Additionally, asymmetric gait is expected where foot strikes are equidistant in space, i.e., the step length is approximately 50% of the stride length. The shortened stride length is a characteristic of motor disorders due to basal ganglia dysfunction in mice [30].

Present data showed an asymmetric gait pattern for mutant mice. However, shortened stride and step lengths and widened step width for both hind limbs may indicate bilateral impairment in which mutants were using their limbs for balance. Likewise, tremor mice showed lower velocity than WT mice [24]. Hypokinesia (reduced gait velocity) with a shortened stride length was described in basal ganglia and striatal degenerative processes such as Parkinson's and Huntington's disease [30]. Thus, given these experiments, the mutant mice had progressive motor impairment until 70 days of age.

Some mutant mice presenting tremors also show hypomyelination/demyelination [6]. To investigate possible central mechanisms related to the motor impairment of the mutants, the GFAP immunohistochemistry in astrocytes of the frontal cortex and mesencephalon, myelin integrity in the white matter of the mesencephalon, cerebellum, and spinal cord were assessed. The present results did not indicate a role of astrocytes or myelin morphology, discarding a neurodegenerative mechanism underlying the impairment of motor skills of the mutant. Also, the lack of effects on cytokines levels revealed that inflammatory processes are not involved with the tremor mice impairments.

Our previous studies about the neurotransmitters in tremor mice [4] showed higher hippocampal gene expression of Egr3 and Gabra1 and increased concentrations of NOR, 5-HT, 5-HIAA, GABA, glutamate, and aspartate. These data associated the audiogenic seizures of tremor mice hippocampal alterations of the Egr3 and Gabra1 gene expression and amino acid and monoamine content. In contrast, the glycine content and the vanillylmandelic acid (VMA)/NOR ratio decreased. Regarding the dopaminergic system, neither DA and DOPAC contents nor the turnover rate of DA showed statistically significant differences between WT and mutant mice.

In the present study, the levels of monoamines and amino acids were evaluated in other central nervous areas as the frontal cortex, striatal, and cerebellum, to investigate the possible involvement of these neurotransmitters in these brain areas with mutant motor disturbances [31–33]. The striatum, a basal ganglia component, facilitates voluntary movement, while the cerebellum maintains the balance and coordination of voluntary movements. Together with the cerebral cortex, both structures mediate the movements, and several neurotransmitters are

involved in the motor control circuitries. DA, 5-HT, GABA, and glutamate mainly regulate the excitation and inhibition of motor neurons. These neurotransmitters control the proper functioning of motor neurons in the striatum and cerebellum [34].

Obeso et al. [34] described that the basal ganglia's motor circuit has two entry points: the striatum and the subthalamic nucleus, and an output, the globus pallidus pars interna, connected to the cortex via the motor thalamus. The neuronal afferents of given movement are coded to the basal ganglia by two different systems: (1) Direct disynaptic projections to the globus pallidus pars interna via the striatum and subthalamic nucleus; (2) Indirect trisynaptic projections to the globus pallidus pars interna via the globus pallidus pars externa. Corticostriatal afferents primarily inhibit medium spiny neurons in the "indirect circuit" and facilitate neurons in the "direct circuit." The globus pallidus pars externa is in a pivotal position to regulate the motor output of the basal ganglia. Dopamine finely tunes striatal input and neuronal activity and modulates the globus pallidus pars externa, globus pallidus pars interna, and the subthalamic nucleus activity.

Dopamine, the principal neurotransmitter related to motor control, can be released in the substantia nigra pars compacta from the dendrites of nigrostriatal dopaminergic neurons [35] to the striatum and throughout the thalamus reach the cortex with excitatory effect on motor activity (direct projection). The release of DA is regulated by a feedback loop to substance nigra articulate where GABA act as a negative control to substance nigra pars compact release dopamine. In addition, all areas of the cerebral cortex forward excitatory glutaminergic impulses to the neostriatum, mainly to the putamen nucleus. From the neostriatum (mainly the putamen nucleus), the axons of GABAergic (inhibitory) neurons emerge towards the globus pallidum nucleus. From this nucleus, GABAergic inhibitory neurons forward their axons towards the thalamic nucleus, structured a morpho-functional mechanism of successive double inhibition, determining, through double inhibition until the cortex. This process of disinhibition of the thalamus towards the cortical areas facilitates the activation of these areas, resulting in great activation in the frontal lobe as the primary motor cortex, pre-motor area, and supplementary motor area.

Our results showed a striatal increase of the dopaminergic system activity (increased HVA/DA ratio) and reduced glycine and aspartate levels without interferences in DA striatal levels. The decreased DA activity in the frontal cortex (DOPAC/DA ratio) and the GABA, glutamate, and aspartate levels suggest reduced inhibitory inputs to this area in the tremor mutant. Thus, the decreased double inhibition may reduce dopamine activity in the frontal cortex and induce tremors, postural instability, and ataxia. In this respect, patients with essential tremors are associated with reduced power across feedback in the motor cortex and reduced connectivity between the parietal and motor cortices [36].

Many of these symptoms are proposed to be due to the disruption of basal ganglia or cerebellar outputs to areas of the cerebral cortex involved in movement control. In the cerebellar cortex, all excitatory afferent signals are subsequently transformed into inhibitory ones. The cerebellar cortex produces an inhibitory influence on these target neurons modifying their excitatory action to activate muscle fibers, which progressively integrate the intended motion. Disturbances that affect this cerebellar inhibition cause uncoordinated decomposed, and ataxic movements, commonly referred to as cerebellar ataxia. While GABA mediates fast inhibitory neurotransmission throughout the CNS, the action of glycine as a fast-inhibitory neurotransmitter is more restricted. Inhibitory glycinergic receptors are particularly relevant for controlling excitability in the mammalian spinal cord, brain stem, and a few selected brain areas, such as the cerebellum and the retina [37].

The GABA hypothesis as a cause for tremor was discarded by our results in mutant mice as there was no difference in cerebellar GABA content between mutant and control mice. It is known that cerebellar neurodegeneration with Purkinje cell loss and decreased cerebellar GABA lead to stimulation of deep cerebellar neurons with pacemaker activity and increased thalamo-cortical rhythmic activity causing tremor [38]. Although glycine is an inhibitory neurotransmitter that is mainly active in the caudal areas of the CNS, it also participates in excitatory neurotransmission since it is a co-agonist of the NMDA subtype of glutamate receptors [39]. So, the increased glycine content in the cerebellum may be related to a persistent overactivity of the glutamatergic system even though glutamate was not increased in the cerebellum of mutant mice.

The role of glycine site ligands in the control of motor behavior seems to be only partially like that of the NMDA receptor ligands, although they bind at the same receptor complex. The glycine site of the NMDA receptor complex has regulatory and modulatory function in the striatum. In these synapses, glycine acts as a co-agonist of glutamate at ionotropic N-methyl-D-aspartate receptors [40]. The output of the motor cortex and the adjacent pre-motor and supplementary cortices is shaped by input from the basal ganglia and cerebellum. Due to the opposite effects of DA and GLU, blockade of NMDA receptors antagonizes the effects of blockade of the DA system that induces rigidity and akinesia, symptoms (Yoshida et al., 1991). Per se, this blockade increases motor activity and induces stereotyped sniffing behavior, head waving, locomotion and a prepulse inhibition deficit of the acoustic startle response [41,42]. The tremor mutant showed increased cerebellar glycine and decreased striatal glycine. Thus, the reduced striatal and increased cerebellar glycine levels could at least be partially responsible for the mutant tremor motor disturbances.

In the motor system, 5-HT is found to either enhance or depress glutamate-mediated transmission and GABA-mediated transmission in structures controlling movement [43]. A recent study published by our research team has shown raised levels of 5-HT and its metabolite 5-HIAA in the hippocampus of tremor mutant [4]. In agreement with our findings, other authors described higher baseline contents of 5-HT and its metabolite in the temporal cortex, hippocampus, and medulla of Krushinsky–Molodkina (KM) rats than in control Wistar rats [44] and the hippocampus of Egr3-deficient mice [45]. 5-HT is generally involved in the mediation of motor behavior through the cerebellum. Serotonin innervations from other motor structures also influence the cerebellum to modulate motor behavior [43].

Serotonin acts upon excitatory transmission in several functions, including motor control [46] and as a mediator in inflammatory processes [47]. 5-HT neurons are widely dispersed in the raphe nuclei of the brain stem, such as the pons and medulla oblongata, and other brain regions, such as the striatum, hippocampus, amygdala, cerebral cortex, thalamus, hypothalamus, and spinal cord [48]. At the striatum, we observed increased 5-HIAA levels and 5-HIAA /5-HT ratio, meaning the increased activity of serotoninergic striatal levels. Thus, a possible contribution of the serotoninergic striatal system in the tremor of our mutant mice cannot be discharged.

Concerning the effects of serotonin as a mediator of the inflammatory process, our data from peripheral cytokines levels did not reveal peripheral inflammatory processes in the mutant tremors. However, Garcia-Gomes et al. [5] showed that the expression of Il6 cytokine decreased and increased the CCL3 chemokine expression in the hippocampus of naïve tremor mice. After audiogenic seizures, the tremor mice showed decreased Ccl3 and Il-1β mRNA expression, while Il6 was increased relative to the naïve this mutant had. However, our results about the GFAP expression of astrocytes in the frontal cortex and mesencephalon did not reveal signs of neuroinflammation in these central areas. The lack of differences relative to myelin sheaths in the white matter of the mesencephalon, cerebellum, and spinal cord here, discards the involvement of myelin changes in the motor dysfunction of tremor mice. Thus, we propose that peripheral and central inflammatory processes are not related to the behavioral and neurobiological impairments in motor dysfunction of tremor mice.

We observed increased NOR levels in cerebellum with a decreased frontal VMA/NOR ratio, meaning a reduced noradrenergic system activity in the mutant mice. The noradrenaline metabolite in the striatum was undetectable.

Concerning the cerebellum, this area receives a noradrenergic pathway from the locus coeruleus. Few studies are about the noradrenergic system and tremor. In tremor induced by harmaline, the noradrenergic innervation of the cerebellum interacts with cerebral serotonergic systems and plays an inhibitory role in the tremor model [49]. The most evidence of noradrenaline involvement in essential tremor is based on the effectiveness of beta-adrenergic antagonists in ameliorating essential tremor [50–52]. Moreover, some evidence proposed that both peripheral and central mechanisms involved the noradrenergic system with tremor [53,54]. More studies are necessary about the role of the noradrenergic system on motor dysfunction observed in tremor mice.

5. Conclusions

We suggest that the motor disturbances resulted mainly from the activation of the indirect striatal inhibitory pathway to the frontal cortex mediated by GABA, glutamate, and aspartate, reducing the dopaminergic activity at the prefrontal cortex, which was associated with the progressive tremor. The reduced striatal and increased cerebellar glycine levels could at least be partially responsible for the mutant tremor motor disturbances.

CRediT authorship contribution statement

Conceptualization, Project administration-M.M. Bernardi, FB Gonçalves; CM Mori. Data acquisition: FB Gonçalves, AC. Silva-Sampaio, TM Sandini, EF Bondan; Mariana M.S.A. Garcia-Gomes; I. Lebrun, JC. Florio, SMG Massironi, SR Alexandre-Ribeiro. Writing – review & editing: MM Bernardi, EF Bondan, AC. Coque, TB. Kirsten.

Conflict of Interest Statement

The authors declare no conflict of interest.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2023.114329.

References

[1] M. Hallett, Tremor: pathophysiology, Park. Relat. Disord. 20 (2014) S118–S122, https://doi.org/10.1016/S1353-8020(13)70029-4.
[2] F. Gövert, G. Deuschl, Tremor entities and their classification, Curr. Opin. Neurol. 28 (2015) 393–399, https://doi.org/10.1097/WCO.0000000000000211.
[3] R. Elble, G. Deuschl, Milestones in tremor research, Mov. Disord. 26 (2011) 1096–1105, https://doi.org/10.1002/mds.23579.
[4] M.S.A. Garcia-Gomes, D.A. Zanatto, O.Y. Galvis-Alonso, J. Mejia, A.T.F.B. Antiorio, P.K. Yamamoto, M.C.M. Olivato, T.M. Sandini, J.C. Flório, I. Lebrun, S.M. G. Massironi, S.R. Alexandre-Ribeiro, M.M. Bernardi, S. Ienne, T.A. de Souza, M.L. Z. Dagli, C.M.C. Mori, Behavioral and neurochemical characterization of the spontaneous mutation tremor, a new mouse model of audiogenic seizures, Epilepsy Behav. 105 (2020), 106945, https://doi.org/10.1016/j.yebeh.2020.106945.
[5] M. de Souza Aranha Garcia-Gomes, P.K. Yamamoto, S.M.G. Massironi, O.Y. Galvis-Alonso, J. Mejia, D.A. Zanatto, S.R. Alexandre-Ribeiro, S. Ienne, C.M.C. Mori, Alteration of hippocampal Egr3, GABA A receptors, Il-1β, Il6 and Ccl3 expression in audiogenic tremor mice after seizure, Epilepsy Behav. 137 (2022), 108962, https://doi.org/10.1016/j.yebeh.2022.108962.
[6] A. Kondo, H. Nagara, K. Akazawa, J. Tateishi, T. Serikawa, J. Yamada, Cns pathology in the neurological mutant rats zitter, tremor and zitter-tremor double mutant (spontaneously epileptic rat, ser): exaggeration of clinical and neuropathological phenotypes in ser, Brain 114 (1991) 979–999, https://doi.org/10.1093/brain/114.2.979.
[7] A.M. Colangelo, L. Alberghina, M. Papa, Astrogliosis as a therapeutic target for neurodegenerative diseases, Neurosci. Lett. 565 (2014) 59–64, https://doi.org/10.1016/j.neulet.2014.01.014.
[8] D. Boison, C. Steinhäuser, Epilepsy and astrocyte energy metabolism, Glia 66 (2018) 1235–1243, https://doi.org/10.1002/glia.23247.
[9] A. Vezzani, E. Aronica, A. Mazarati, Q.J. Pittman, Epilepsy and brain inflammation, Exp. Neurol. 244 (2013) 11–21, https://doi.org/10.1016/j.expneurol.2011.09.033.
[10] A. Vezzani, A. Friedman, R.J. Dingledine, The role of inflammation in epileptogenesis, Neuropharmacology 15 (2018) 1–12, https://doi.org/10.1016/j.neuropharm.2012.04.004.
[11] A. Gironell, J. Marin-Lahoz, The essence of essential tremor: neurochemical bases, Rev. Neurol. 62 (2016) 507–515. ⟨http://www.ncbi.nlm.nih.gov/pubmed/27222085⟩.
[12] E.D. Louis, Essential tremor and the cerebellum, in: M. Aminoff, F. Boller, D. Swaab (Eds.), Handb. Clin. Neurol., Elsevier B.V., Amsterdam, 2018, pp. 245–258, https://doi.org/10.1016/B978-0-444-64189-2.00016-0.
[13] G.A. Prieto, Abnormalities of dopamine D 3 receptor signaling in the diseased brain, J. Cent. Nerv. Syst. Dis. 9 (2017), 117957351772633, https://doi.org/10.1177/1179573517726335.
[14] M.K. Pan, C.L. Ni, Y.C. Wu, Y.S. Li, S.H. Kuo, Animal models of tremor: relevance to human tremor disorders, Tremor Other Hyperkinetic Mov. 8 (2018) 587, https://doi.org/10.7916/D89S37MV.
[15] N.R. Council, Guide for the Care and Use of Laboratory Animals, Committee for the Updated of the Guide for the Care and Use of Laboratory Animals, 8th, 2010.
[16] P.K. Yamamoto, T.A. Souza, A.T.F.B. Antiorio, D.A. Zanatto, M. de, S.A. Garcia-Gomes, S.R. Alexandre-Ribeiro, N. de, S. Oliveira, C.F.M. Menck, M.M. Bernardi, S. M.G. Massironi, C.M.C. Mori, Genetic and behavioral characterization of a Kmt2d mouse mutant, a new model for Kabuki Syndrome, Genes Brain Behav. 18 (2019), e12568, https://doi.org/10.1111/gbb.12568.
[17] B.Y. Jacobs, H.E. Kloefkorn, K.D. Allen, Gait analysis methods for rodent models of osteoarthritis, Curr. Pain Headache Rep. 18 (2014) 456, https://doi.org/10.1007/s11916-014-0456-x.
[18] V. Bonifati, P. Rizzu, M.J. van Baren, O. Schaap, G.J. Breedveld, E. Krieger, M.C. J. Dekker, F. Squitieri, P. Ibanez, M. Joosse, J.W. van Dongen, N. Vanacore, J. C. van Swieten, A. Brice, G. Meco, C.M. van Duijn, B.A. Oostra, P. Heutink, Mutations in the DJ-1 gene associated with autosomal recessive early-onset Parkinsonism, Science (80) (299) (2003) 256–259, https://doi.org/10.1126/science.1077209.
[19] Y. Ohno, S. Shimizu, A. Tatara, T. Imaoku, T. Ishii, M. Sasa, T. Serikawa, T. Kuramoto, Hcn1 is a tremorgenic genetic component in a rat model of essential tremor, PLoS One 10 (2015), e0123529, https://doi.org/10.1371/journal.pone.0123529.
[20] X. Mao, P. Ma, D. Cao, C. Sun, Z. Ji, D. Min, H. Sun, N. Xie, J. Cai, Y. Cao, Altered expression of GABAA receptors (α4, γ2 subunit), potassium chloride cotransporter 2 and astrogliosis in tremor rat hippocampus, Brain Res. Bull. 86 (2011) 373–379, https://doi.org/10.1016/j.brainresbull.2011.09.002.
[21] M.C. La Regina, K. Yates-Siilata, L. Woods, D. Tolbert, Preliminary characterization of hereditary cerebellar ataxia in rats, Lab. Anim. Sci. 42 (1992) 19–26. ⟨http://www.ncbi.nlm.nih.gov/pubmed/1316503⟩.
[22] M. Dierssen, V. Fotaki, M. Martínez de Lagrán, M. Gratacós, M. Arbonés, C. Fillat, X. Estivill, Neurobehavioral development of two mouse lines commonly used in transgenic studies, Pharm. Biochem. Behav. 73 (2002) 19–25, https://doi.org/10.1016/S0091-3057(02)00792-X.
[23] Y. Jiao, J. Yan, Y. Zhao, L.R. Donahue, W.G. Beamer, X. Li, B.A. Roe, M.S. LeDoux, W. Gu, Carbonic anhydrase-related protein VIII deficiency is associated with a distinctive lifelong gait disorder in waddles mice, Genetics 171 (2005) 1239–1246, https://doi.org/10.1534/genetics.105.044487.
[24] M.S.A. Garcia-Gomes, D. Zanatto, P. Yamamoto, D. Wadt, A. Antiorio, J. Aleman-Laporte, S. Alexandre-Ribeiro, G. Marson, C. Guizzo, S. Massironi, M. Bernardi, C. Mori, A simple and fast battery test for phenotypic characterization of mice, Bio-Protocol 10 (2020), e3568, https://doi.org/10.21769/BioProtoc.3568.
[25] S. Dutta, P. Sengupta, Men and mice: relating their ages, Life Sci. 152 (2016) 244–248, https://doi.org/10.1016/j.lfs.2015.10.025.
[26] A.V. Kalueff, J.W. Aldridge, J.L. LaPorte, D.L. Murphy, P. Tuohimaa, Analyzing grooming microstructure in neurobehavioral experiments, Nat. Protoc. 2 (2007) 2538–2544, https://doi.org/10.1038/nprot.2007.367.
[27] J. Altman, K. Sudarshan, Postnatal development of locomotion in the laboratory rat, Anim. Behav. 23 (1975) 896–920, https://doi.org/10.1016/0003-3472(75)90114-1.
[28] G.R. Finney, Perceptual-motor dysfunction, Continuum 21 (2015) 678–689, https://doi.org/10.1212/01.CON.0000466660.82284.69.
[29] T.N. Luong, H.J. Carlisle, A. Southwell, P.H. Patterson, Assessment of motor balance and coordination in mice using the balance beam, J. Vis. Exp. 49 (2011) 2376, https://doi.org/10.3791/2376.
[30] P.O. Fernagut, E. Diguet, B. Labattu, F. Tison, A simple method to measure stride length as an index of nigrostriatal dysfunction in mice, J. Neurosci. Methods 113 (2002) 123–130, https://doi.org/10.1016/S0165-0270(01)00485-X.
[31] J. Yoshida, M. Oñate, L. Khatami, J. Vera, F. Nadim, K. Khodakhah, Cerebellar contributions to the Basal Ganglia influence motor coordination, reward

processing, and movement vigor, J. Neurosci. 42 (2022) 8406–8415, https://doi.org/10.1523/JNEUROSCI.1535-22.2022.
[32] M. Novello, L.W.J. Bosman, C.I.De Zeeuw, A systematic review of direct outputs from the cerebellum to the brainstem and diencephalon in mammals, Cerebellum (2022), https://doi.org/10.1007/s12311-022-01499-w.
[33] M.J. Wagner, T.H. Kim, J. Savall, M.J. Schnitzer, L. Luo, Cerebellar granule cells encode the expectation of reward, Nature 544 (2017) 96–100, https://doi.org/10.1038/nature21726.
[34] J.A. Obeso, M.C. Rodríguez-Oroz, B. Benitez-Temino, F.J. Blesa, J. Guridi, C. Marin, M. Rodriguez, Functional organization of the basal ganglia: therapeutic implications for Parkinson's disease, Mov. Disord. 23 Suppl. 3 (2008) S548–S559, https://doi.org/10.1002/mds.22062.
[35] A. Cheramy, V. Leviel, J. Glowinski, Dendritic release of dopamine in the substantia nigra, Nature 289 (1981) 537–543, https://doi.org/10.1038/289537a0.
[36] A. Roy, S.A. Coombes, J.W. Chung, D.B. Archer, M.S. Okun, C.W. Hess, A. Wagle Shukla, D.E. Vaillancourt, Cortical dynamics within and between parietal and motor cortex in essential tremor, Mov. Disord. 34 (2019) 95–104, https://doi.org/10.1002/mds.27522.
[37] P. Legendre, The glycinergic inhibitory synapse, Cell. Mol. Life Sci. 58 (2001) 760–793, https://doi.org/10.1007/pl00000899.
[38] A. Gironell, The GABA hypothesis in essential tremor: lights and shadows, Tremor Other Hyperkinetic Mov. 4 (2014) 254, https://doi.org/10.5334/tohm.229.
[39] F. Zafra, C. Giménez, Glycine transporters and synaptic function, IUBMB Life 60 (2008) 810–817, https://doi.org/10.1002/iub.128.
[40] J.W. Johnson, P. Ascher, Glycine potentiates the NMDA response in cultured mouse brain neurons, Nature 325 (1987) 529–531, https://doi.org/10.1038/325529a0.
[41] W.J. Schmidt, Intrastriatal injection of dl-2-amino-5-phosphonovaleric acid (AP-5) induces sniffing stereotypy that is antagonized by haloperidol and clozapine, Psychopharmacology 90 (1986), https://doi.org/10.1007/BF00172883.
[42] A. Imperato, M.G. Scrocco, S. Bacchi, L. Angelucci, NMDA receptors and in vivo dopamine release in the nucleus accumbens and caudatus, Eur. J. Pharm. 187 (1990) 555–556, https://doi.org/10.1016/0014-2999(90)90387-L.
[43] D.Y. Abg Abd Wahab, C.H. Gau, R. Zakaria, M.K. Muthu Karuppan, B.S. A-Rahbi, Z. Abdullah, A. Alrafiah, J.M. Abdullah, S. Muthuraju, Review on cross talk between neurotransmitters and neuroinflammation in striatum and cerebellum in the mediation of motor behaviour, BioMed Res. Int. 2019 (2019) 1767203, https://doi.org/10.1155/2019/1767203.
[44] I.I. Poletaeva, N.M. Surina, Z.A. Kostina, O.V. Perepelkina, I.B. Fedotova, The Krushinsky-Molodkina rat strain: the study of audiogenic epilepsy for 65 years, Epilepsy Behav. 71 (2017) 130–141, https://doi.org/10.1016/j.yebeh.2015.04.072.
[45] A. Gallitano-Mendel, D.F. Wozniak, E.A. Pehek, J. Milbrandt, Mice lacking the immediate early gene Egr3 respond to the anti-aggressive effects of clozapine yet are relatively resistant to its sedating effects, Neuropsychopharmacology 33 (2008) 1266–1275, https://doi.org/10.1038/sj.npp.1301505.
[46] B.L. Jacobs, E.C. Azmitia, Structure and function of the brain serotonin system, Physiol. Rev. 72 (1992) 165–229, https://doi.org/10.1152/physrev.1992.72.1.165.
[47] R. Mössner, K.P. Lesch, Role of serotonin in the immune system and in neuroimmune interactions, Brain Behav. Immun. 12 (1998) 249–271, https://doi.org/10.1006/brbi.1998.0532.
[48] Y. Ohno, S. Shimizu, K. Tokudome, N. Kunisawa, M. Sasa, New insight into the therapeutic role of the serotonergic system in Parkinson's disease, Prog. Neurobiol. 134 (2015) 104–121, https://doi.org/10.1016/j.pneurobio.2015.09.005.
[49] W. Kolasiewicz, K. Kuter, P. Nowak, A. Pastuszka, K. Ossowska, Lesion of the cerebellar noradrenergic innervation enhances the harmaline-induced tremor in rats, Cerebellum 10 (2011) 267–280, https://doi.org/10.1007/s12311-011-0250-9.
[50] W.C. Koller, B. Vetere-Overfield, Acute and chronic effects of propranolol and primidone in essential tremor, Neurology 39 (1989) 1587–1588, https://doi.org/10.1212/wnl.39.12.1587.
[51] J. Mally, T.W. Stone, Efficacy of an adenosine antagonist, theophylline, in essential tremor: comparison with placebo and propranolol, J. Neurol. Sci. 132 (1995) 129–132, https://doi.org/10.1016/0022-510X(95)00128-O.
[52] A. Gironell, J. Kulisevsky, M. Barbanoj, D. López-Villegas, G. Hernández, B. Pascual-Sedano, A randomized Placebo-controlled comparative trial of gabapentin and propranolol in essential tremor, Arch. Neurol. 56 (1999) 475–480, https://doi.org/10.1001/archneur.56.4.475.
[53] B. Abila, J.F. Wilson, R.W. Marshall, A. Richens, The tremorolytic action of beta-adrenoceptor blockers in essential, physiological and isoprenaline-induced tremor is mediated by beta-adrenoceptors located in a deep peripheral compartment, Br. J. Clin. Pharm. 20 (1985) 369–376, https://doi.org/10.1111/j.1365-2125.1985.tb05079.x.
[54] I.-U. Song, S.-W. Ha, Y.-S. Yang, Y.-A. Chung, Differences in regional glucose metabolism of the brain measured with F-18-FDG-PET in patients with essential tremor according to their response to beta-blockers, Korean J. Radio. 16 (2015) 967–972, https://doi.org/10.3348/kjr.2015.16.5.967.